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Doctoral Dissertation

從耳石日成長輪及微化學探討西北太平洋區鱸鰻之生活史特性

LIFE HISTORY TRAITS OF THE GIANT MOTTLED EEL *ANGUILLA MARMORATA* IN THE NORTHWESTERN PACIFIC AS REVEALED FROM OTOLITH DAILY GROWTH INCREMENT AND MICROCHEMISTRY

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Life history traits of the giant mottled eel *Anguilla marmorata* in the Northwestern Pacific as revealed from otolith daily growth increment and microchemistry

本論文係林德君（學號 D98B45005）在國立臺灣大學漁業科學研究所完成之博士學位論文，於民國 103 年 06 月 27 日承下列考試委員審查通過及口試及格，特此證明

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摘要

為了瞭解西北太平洋熱帶性鱸鰻之加入動態和初期生活史以及菲律賓水域同種鰻的洄游環境史。本研究調查2005-2009年台灣東部秀姑巒溪河口玻璃鰻的種類組成，結果發現鱸鰻的玻璃鰻數量最多，占總捕獲量的98.4%；另外有少量的太平洋雙色鰻 (1.6%) 及日本鰻(<1%)。熱帶性鱸鰻的玻璃鰻的主要出現季節為春夏天，但幾乎終年都可發現；而太平洋雙色鰻的玻璃鰻的主要出現季節為秋天；溫帶性日本鰻的玻璃鰻的出現季節則是在冬天。

透過東北亞地區不同河口所捕獲的鱸鰻和日本鰻玻璃鰻的耳石日周輪分析顯示，柳葉鰻變態的日齡及早期成長率對於此兩種同域分布鰻魚的分離洄游及緯度分布扮演重要的角色。成長快、變態早的鱸鰻柳葉鰻較早加入到菲律賓；而成長慢、延遲變態的日本鰻柳葉鰻繼續向南(經由明答那峨海流)及向北(經由黑潮)飄送。另一方面，日本鰻柳葉鰻抵達菲律賓水域時尚未到變態階段，所以也不會洄游到河口，而是繼續向北散佈。這可能就是日本鰻很少在菲律賓發現而鱸鰻在菲律賓數量很多的原因。

電子微探儀(EPMA)分析顯示，採集於菲律賓呂宋島東部河川的鱸鰻耳石鍶鈣比數據和之前所報導的日本及越南的鱸鰻以及日本鰻的洄游環境史非常不同。在耳石鰻線標記之後到耳石邊緣的鍶鈣比都低於4×10^{3}，顯示菲律賓呂宋島東側的鱸鰻在鰻線階段進入淡水溪流後一直到被捕獲的黃鰻階段為止都悽息在淡水環境中。此結果和台灣的鱸鰻一，在黃鰻階段都只棲息在淡水中。而日本鰻在黃鰻階段的棲地利用則較為彈性，可在海水、鹹淡水和淡水之間洄游。種間競爭、環境喜好性以及生產力可能是造成鱸鰻和日本鰻的棲地喜好性不同的原因。本研究之發現可提供鰻魚資源保育之參考。

關鍵字：耳石、日成長輪、鱸鰻、玻璃鰻、加入動態、洄游環境史

ABSTRACT

The present study investigated the recruitment dynamics and early life history of the tropical eel *Anguilla marmorata* in the northwestern Pacific as well as the migratory environmental history of the eel in the Philippine waters.

Analysis of the species composition of the recruiting glass eels in the estuary of the Hsiukuluan River, Eastern Taiwan from 2005-2009 revealed that *A. marmorata* was the most dominant eel species making up to 98.4% of the total catch while there were very few *A. bicolor pacifica* (1.6%) and *A. japonica* (<1%). Tropical eel species *A. marmorata* recruited mainly to the estuary during spring to summer but can be found year-round while *A. bicolor pacifica* recruited mainly during autumn. The temperate species, *A. japonica*, recruited mainly during winter.

Examinations of the otolith daily growth increments of *A. marmorata* and *A. japonica* glass eels collected from various rivers and estuaries in East Asia from 1992-2008 indicated that age at metamorphosis and early growth rate seem to play an important role in the segregative migration and latitudinal distribution of these two sympatric eel species in the northwestern Pacific. Faster-growing and earlier-metamorphosing leptocephali of *A. marmorata* recruited earlier in the Philippines while its slower-growing, delayed metamorphosing leptocephali dispersed southward (via the Mindanao Current) and northward (via the Kuroshio Current). On the other hand, the *A. japonica* leptocephali which arrive in the Philippine waters are apparently too young to metamorphose and migrate towards the estuaries so it will continue to drift northwards. This must be the reason why Japanese eels are seldom found in the Philippines while *A. marmorata* occurs in abundance.

The Sr:Ca profile in the otoliths of yellow-stage *A. marmorata* collected in the river of eastern Luzon, the Philippines in August 2008 revealed that it's migratory environmental history

is quite different from that previously reported from Japan and Vietnam and from *A. japonica*. Electron probe microanalyzer showed that after the elver check, the Sr:Ca ratio until the otolith edge were less than 4 x 10^{-3}, indicating that after recruitment, *A. marmorata* just stayed in freshwater until capture, which is similar to that of *A. marmorata* in Taiwan. On the contrary, *A. japonica* has a more flexible migratory behavior in the yellow stage. It can migrate among seawater, brackish water and freshwater in the yellow eel stage. Interspecific competition, environmental factors and the productivity of the environment may play an important role in the habitat preference of *A. marmorata* throughout its species range. The findings of this study can provide the information for the eel conservation and management.

Keywords: Otolith, daily growth increment, giant mottled eel, glass eels, recruitment dynamics, migratory environmental history

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1. INTRODUCTION

1.1 Life history pattern of freshwater eel (*Anguilla* spp.)

Anguillid eels (Genus *Anguilla*) are catadromous fishes, spending the majority of their life cycle in freshwater or estuaries until sexual maturity and migrating to the open ocean to spawn and die (Fig. 1). These long migrations to spawn far offshore appeared to be unique among the >800 anguilliform species (Miller 2009). At the moment, only the spawning grounds of four freshwater eel species in both the Atlantic and the Pacific Oceans have been identified (Schmidt 1922; Tsukamoto 1992; Kuroki et al. 2009). The spawning areas of the other freshwater eel species around the world are still not well understood, thus research interest in eel migration is still focused on the actual spawning location for anguillid eels. Interestingly, these eels in both the Atlantic and the Pacific spawn in similar westward flowing currents at the southern edges of the subtropical gyres in both oceans so that their leptocephali can be passively transported to their respective recruitment areas where they feed and grow (Tsukamoto et al. 2002). At the end of their long transoceanic migration, the leptocephali metamorphose into glass eels and invade coastal and inland habitats. The eels undergo two major metamorphoses throughout their long lives: the first one is the metamorphosis from leptocephalus into glass eel during their migration from their offshore marine spawning ground to their continental freshwater growth habitat. Otolith microchemistry studies have revealed that the glass eels after reaching coastal waters may either migrate further inland and colonize freshwater habitats or stop their migration and settle in seawater or estuary (Tzeng et al. 2002; Arai et al. 2004; Daverat et al. 2006). The timing of the metamorphosis by a leptocephalus into glass eel and the transportation of the oceanic currents are considered to be the key determinant of the ultimate destination of the eel (Cheng and Tzeng 1996; Wang and Tzeng 2000; Tzeng 2003). The second metamorphosis on the other hand, is from yellow eel to silver eel in a process called silvering

and it occurs during their downstream migration from their continental freshwater habitat to their offshore spawning ground in the open ocean. During silvering, their body colouration changes, their swim bladder changes and their eyes enlarge, all of which would facilitate the new demands of deep-water swimming, predator avoidance and possibly visual mate location (Rousseau et al. 2002; Aoyama and Miller 2003). These series of metamorphoses in yellow eels in both freshwater and coastal marine habitats are accompanied by drastic morphological, physiological and behavioural changes to fit their habitat shift and activities associated with a long migration in the open ocean (Bruijs and Durif 2002). Recent study by Tsukamoto et al. (2011) reported that the morphological condition of spawning adults suggests that freshwater eels have the capacity for multiple spawning during a spawning season. However, their highly modified bodies and degenerated condition confirm that they have only one spawning season in their life and die after spawning a few times.

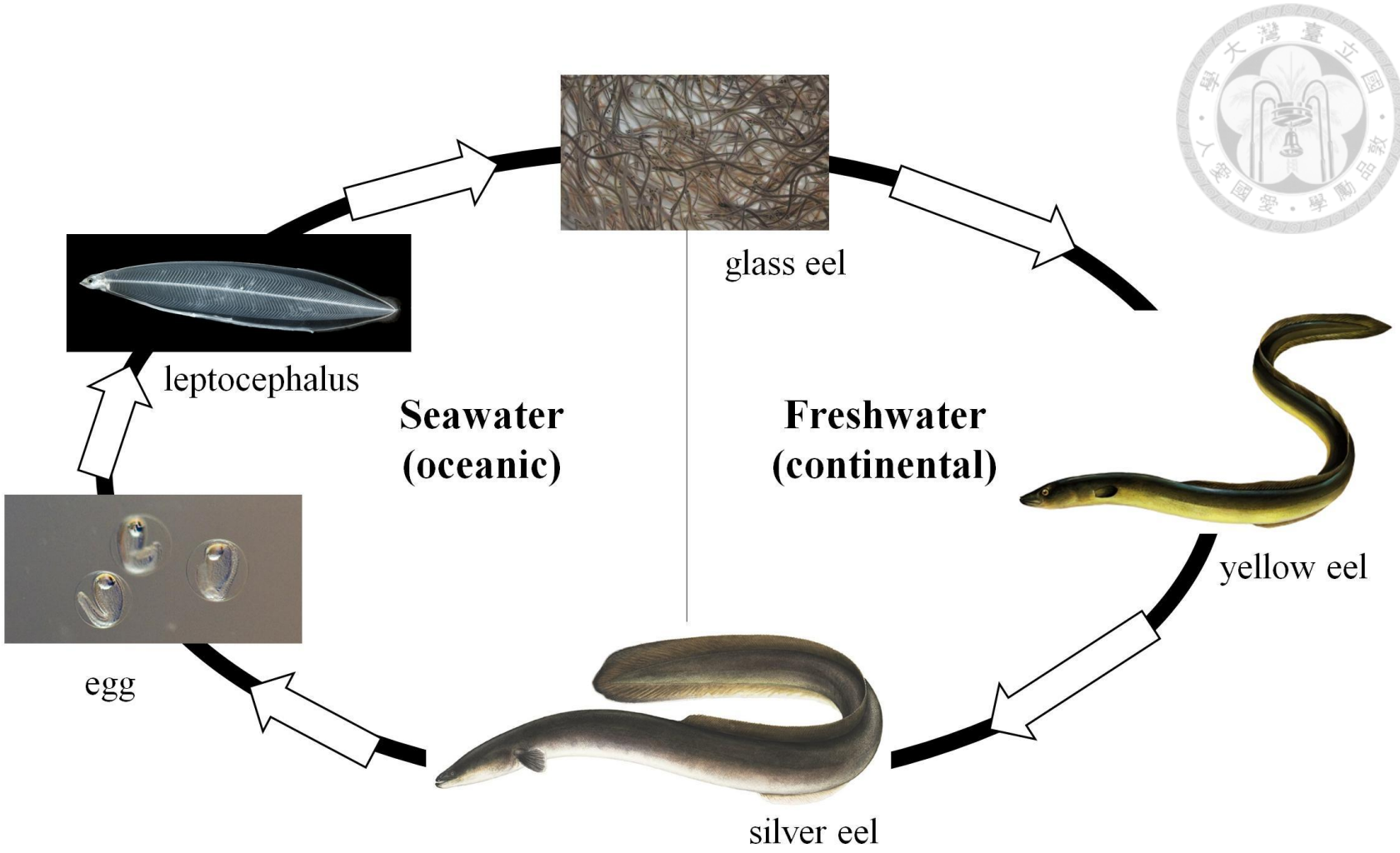


Fig. 1. Life cycle and catadromous migration of anguillid eels.

1.2 Freshwater eel fishery and its problem

Anguillid eels are an economically important fish species in many regions of the world particularly in Europe and East Asia where there is a long standing tradition of its consumption. The Japanese consume around 100, 000 tons of eels every year, of which more than 99.5% are farmed eels (FAO 2012 Statistics). Intensive commercial culture of anguillid eels (particularly *A. japonica*) is an important industry not only in Japan where it was first initiated but also in China, Taiwan and Korea. In fact in recent years, imports of farmed eels from these two countries have grown considerably that they now comprise around 70% of all eels consumed in Japan. The seeds needed for aquaculture production still rely on wild-caught glass eels but because the glass eel resources are in sharp decline globally, the eel farming industry is at a critical point. To ease the reliance for wild-caught eels for aquaculture production and to help protect the wild populations, researches on technology for producing artificial glass eels were initiated. But at the moment, problems such as poor egg quality, appropriate food for the larvae and high density rearing method for leptocephalus among other things, have hindered the completion of its artificial production.

1.3 Population decline of freshwater eel

The world's eel resources have been in sharp decline since the second half of the last century (Fig. 2). In the Pacific Ocean, the annual catch of the Japanese eels is reported to be fluctuating with a continuously decreasing trend (Tatsukawa 2003). Its decline started in around 1970 and it now stand at about 10% of the levels seen in the 1960's. This decreasing trend however is not only observed in/limited to the Japanese eel. In the Atlantic Ocean, European and American eel recruitment is down to less than 1% of its peak in some areas (Richkus and Whalen 2009; Dekker 2008). South Pacific eels are also reported to be in decline (Jellyman et al. 2000;

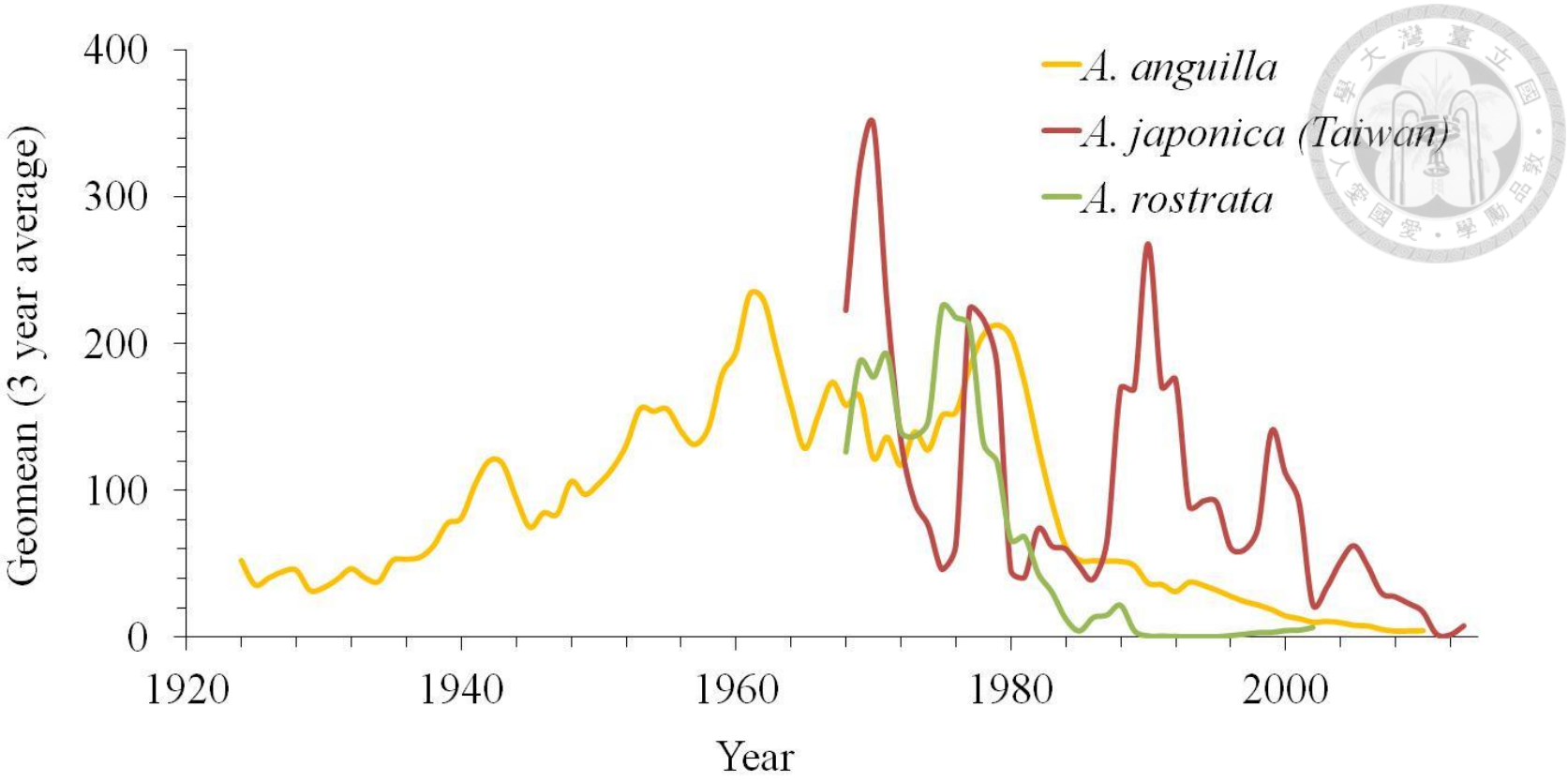


Fig. 2. Recruitment (3 year running averages of geomeans of indices as % of 1979-1994 means) for the European (Moses-Saunders Index – 7 years), American (adjusted to year of arrival) and Japanese glass eels from Taiwan.

Hoyle and Jellyman 2002). The reasons for the decline are unclear but are probably due to spawning stocks reduction (Clevestam et al. 2011), swim bladder parasites and virus infection (Haenen et al. 2002; Szekely et al. 2002; Kirk 2003; Sures and Knopf 2004; van Ginniken et al. 2005) , overfishing (Moriarty and Dekker 1997; Briand et al. 2003; Dekker 2003), growth habitat reduction (McCleave 2001), pollution (Amiard-Triquet et al. 1988; Robinet and Feunteun 2002; van Ginneken et al. 2009), global climate change (Castonguay et al. 1994; Kimura et al. 2001; Knights 2003; Friedland et al. 2007; Bonhommeau et al. 2008) and solar cycle (Tzeng et al. 2012). Previous researches also highlighted the environmental factors in the estuaries such as temperature, salinity, turbidity, pH, stream water odour and tidal cycle as well as moon phase and rainfall, may act alone or in combination to influence recruitment (Sloane 1984; Tzeng 1985; Sorensen and Bianchini 1986; Tosi et al. 1990; Chen Lee et al. 1994).

In the northwestern Pacific, intense fishing pressure in adult *A. japonica* and *A. marmorata* and the widespread harvesting of their glass eels for aquaculture caused their population to decline (Fig. 3). Also, constructions along the river systems like dams and other water impoundments impeded the downstream migration of the adult eels towards the sea and the upstream migration of elvers, further affecting the dwindling eel population. It is because of this that the Taiwanese government was prompted to impose fishing and aquaculture ban on *A. marmorata* and listed it as an endangered species according to the Wildlife Conservation Act of Taiwan. More recently, the Philippine government, through its Bureau of Fisheries and Aquatic Resources (BFAR) issued Fisheries Administrative Order (FAO) No. 242 series of 2012 repealing FAO 159 series of 1986 that allows elver exportation and reinstate the ban on the exportation of live elvers caught in the Philippine waters in a bid to stop the rapid and rampant

exploitations of the anguillid eels in the country (Appendix I). According to BFAR regional office in northern

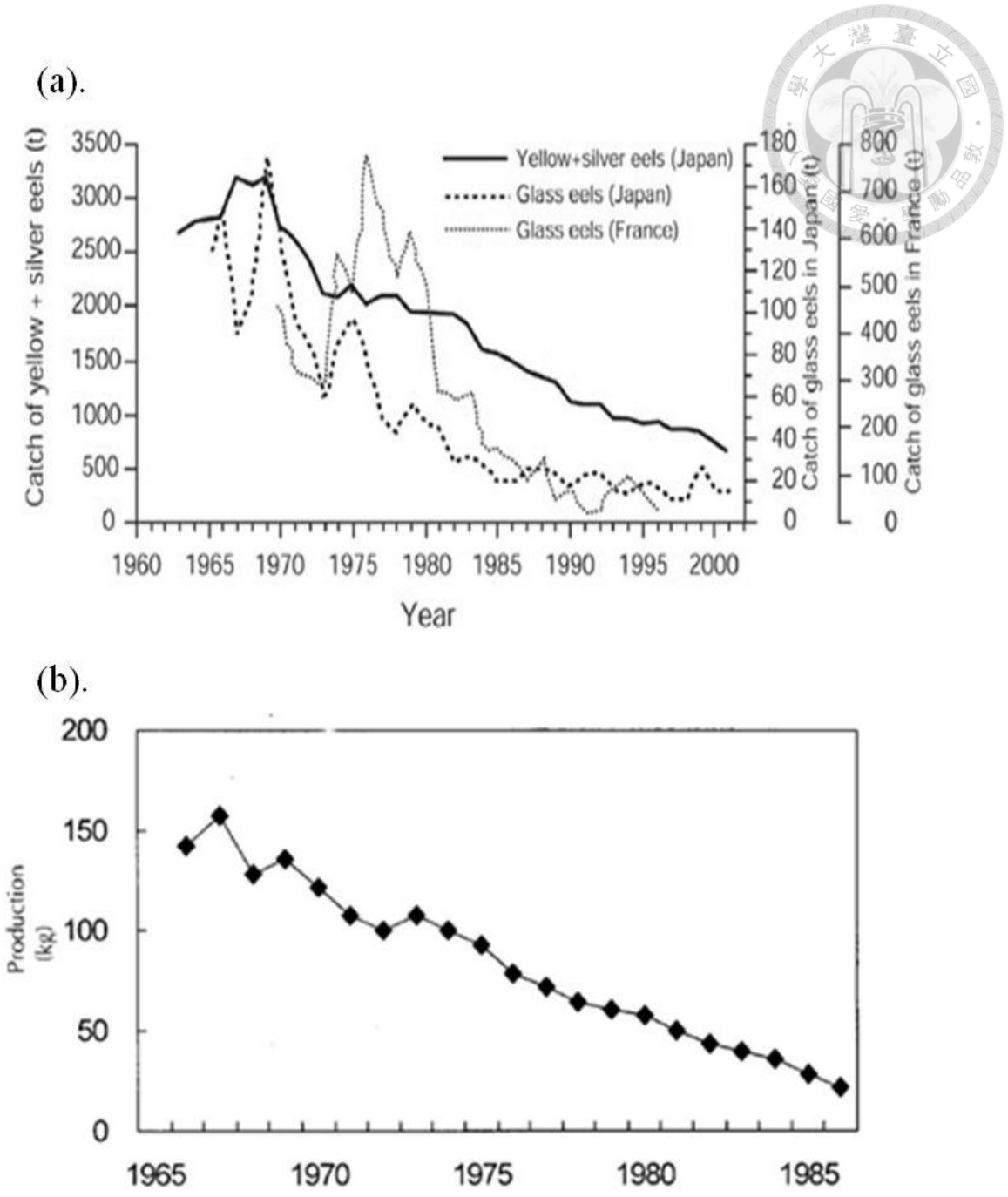


Fig. 3. Population decline in *Anguilla japonica* (a; Tsukamoto et al. 2009) and *A. marmorata* (b; Prof. C.S. Tzeng unpublished).

Philippines, approximately more than 1 million elvers were caught every day during the main fishing season (November to February every year) and from PhP 2,500 (US$ 59.19 or NT$ 1,766.09) per kilo (approx. 5-6 thousand elvers) in 2012, the buying price suddenly went up to 17 thousand (US$ 402.31or NT$ 12,009.43) to 28 thousand (US$ 622.58 or US$ 19,783.14) PhP from January to March 2013. Because of the high demand and price for the elvers, indiscriminate and undocumented elver collections were reported in northern Philippines specifically near the river mouth of Cagayan River. The FAO carries a penalty of eight years imprisonment, confiscation of catch or a fine equivalent to double the export value of the same, and revocation of fishing and/or export permit. In recent years, *A. marmorata* is rapidly gaining popularity as an aquaculture species in East Asia due to the declining supply of *A. japonica* and the soaring prices of its glass eels. *Anguilla marmorata* glass eels are still in abundant supply and its price is much cheaper than those of *A. japonica* glass eels. Despite the ban the Philippines is still supplying increasingly large quantities of *A. marmorata* glass eels caught in major river systems in northern Luzon and in Mindanao, to numerous eel farms in East Asia and are now increasingly being used in many eel farms in China, Taiwan and Korea. Early this year, the International Union on the Conservation of Nature listed *A. japonica* as an endangered species due to a number of factors that includes overfishing and loss of habitat (IUCN, 2014). Because of this, it is expected that *A. marmorata* is poised to take-over *A. japonica* as the major eel species for aquaculture in East Asia. But to prevent *A. marmorata* from following the same fate as A. *japonica*, understanding its life history will help in formulating regulatory measures for its conservation and sustainable use.

1.4 Biology and population structure of *Anguilla marmorata*

The tropical anguillid eel *Anguilla marmorata* is the most widespread in all of the 16 species and 3 subspecies of freshwater eels in the world (Ege 1939; Watanabe 2003). It is found from the southeast coast of Africa in the Indian Ocean, eastward through the islands like Madagascar and Reunion, northward through Indonesia up to southern Japan and Korea and through the tropical western Pacific, including many small islands in the South Pacific (Tesch 2003). More recently, the occurrence of *A. marmorata* outside of its known distribution range has been reported like in Caroline Islands (Myers and Donaldson 2003; Donovan et al. 2012), Palmyra Atoll (Handler and James 2006) and even farther east in the Galapagos (McCosker et al. 2003). It has been widely documented that this species can live in sympatry with the other tropical and temperate eel species. Recent genetic (mtDNA and microsatellite) and morphological (total number of vertebrae) studies suggested that *A. marmorata* has at least four different regional populations in the whole Indo-Pacific (Ishikawa 1998; Ishikawa et al. 2004; Minegishi et al. 2008; Watanabe et al. 2008; Watanabe et al. 2009a). Such type of regional divergence within the same species can be due to each population having different migration loops or migratory pathways brought about by barriers to migration in the Pacific and Indian Oceans. This causes each population to evolve to have unique or specific migration loops adapted to the geography and hydrology of each area. Therefore, the shift in the migration loop could possibly a step towards speciation in freshwater eels. At the moment with its four to five different regional populations, only one spawning ground of *A. marmorata* has been identified and it is in the north equatorial current (NEC) region of the western North Pacific Ocean in about the same region as the spawning area of *A. japonica* (Kuroki et al. 2009; Tsukamoto et al. 2011). Both *A. japonica* and *A. marmorata* were proposed to be spawned in the waters west of the Mariana Islands in the Pacific Ocean (Fig. 4) (Kuroki et

al. 2009; Tsukamoto et al. 2011). Adult individuals migrate thousands of miles from their freshwater growth habitat in East Asia to this location under starving condition because they do not assimilate marine food sources during their spawning migration (Chow et al. 2010). After hatching, their marine larvae, the leptocephalus were transported westward by the NEC from their spawning ground to the continental shelf of the northwestern Pacific. Furthermore, *A. marmorata* will drift both in the northward flowing Kuroshio Current and the southward flowing Mindanao Current (Fig. 5a) while *A. japonica* will only enter the northward flowing Kuroshio Current that will transport them to East Asia particularly in Taiwan, China, Japan and Korea (Fig. 5b). This indicates that the larvae of *A. marmorata* disperses and recruits to a wider area in the northwestern Pacific region than *A. japonica* (Tsukamoto 1992; Kimura et al. 1994; Miller et al. 2006; Kuroki et al. 2006). Why *A. japonica* larvae only enter the Kuroshio Current Region while *A. marmorata*, after being transported by the NEC, can enter both the northward Kuroshio Current and the southward Mindanao Current that carry them to their recruitment areas in northern Indonesia, the Philippines, Taiwan, eastern China, southern Japan and Korea, is still unclear (Zenimoto et al. 2009). Recently, Han et al. (2012) pointed out that recruitment temperature preference and oceanic current system control the distinct biogeography of *A. japonica* and *A. marmorata*. However aside from these environmental controls, biological factors should also be taken into consideration in explaining this ecological discrepancy but the early life histories of these two species particularly during the oceanic phase are not yet fully understood especially that of *A. marmorata*. Upon reaching the continental waters, it was also found that their distribution in the estuaries was also geographically different. In addition, it was also observed that in the same river system, *A. marmorata* occupied the upper reaches while *A. japonica* occupied the lower reaches (Shiao et al. 2003).

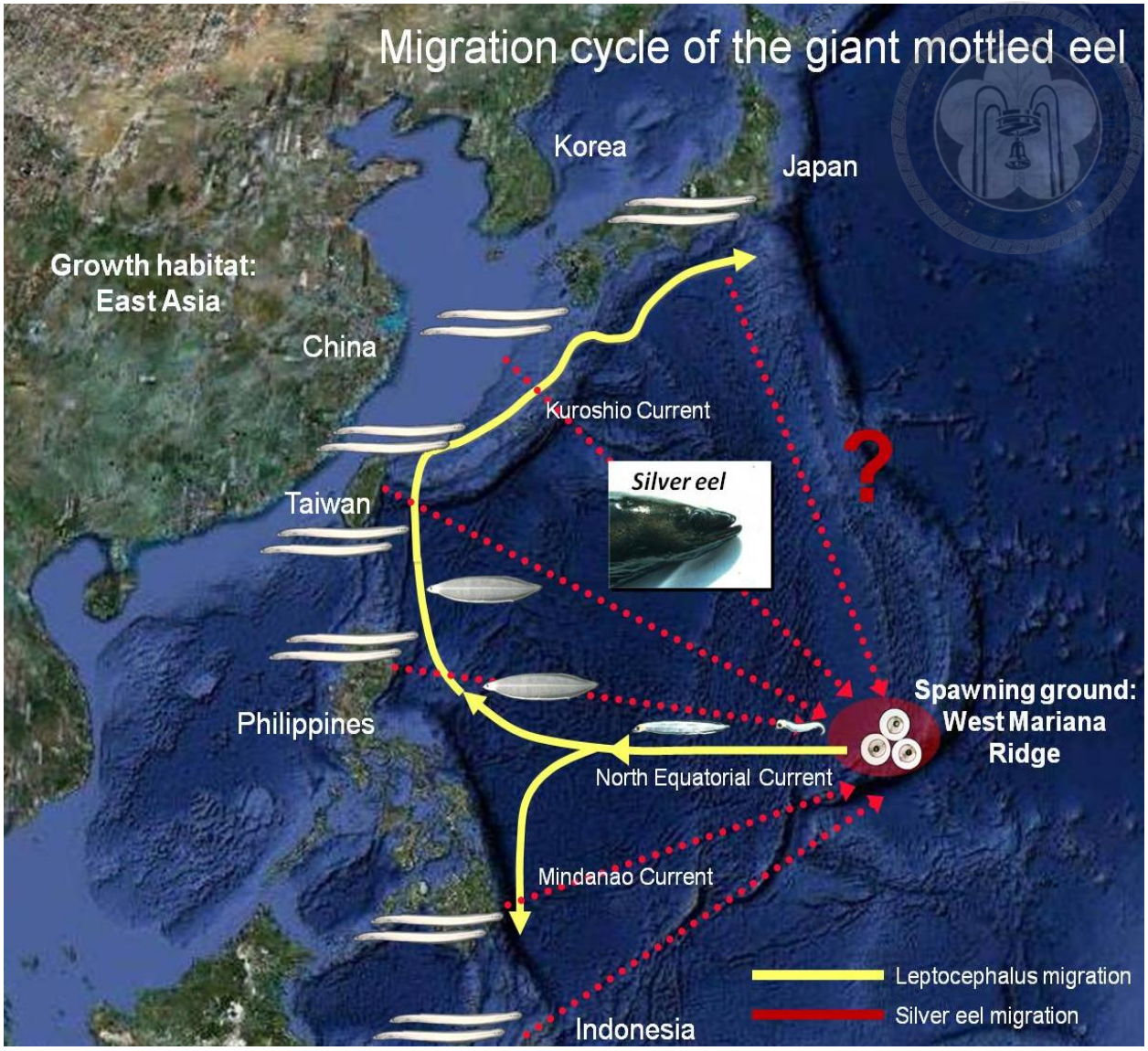


Fig 4. Migration cycle of the northwestern Pacific population of *Anguilla marmorata*.

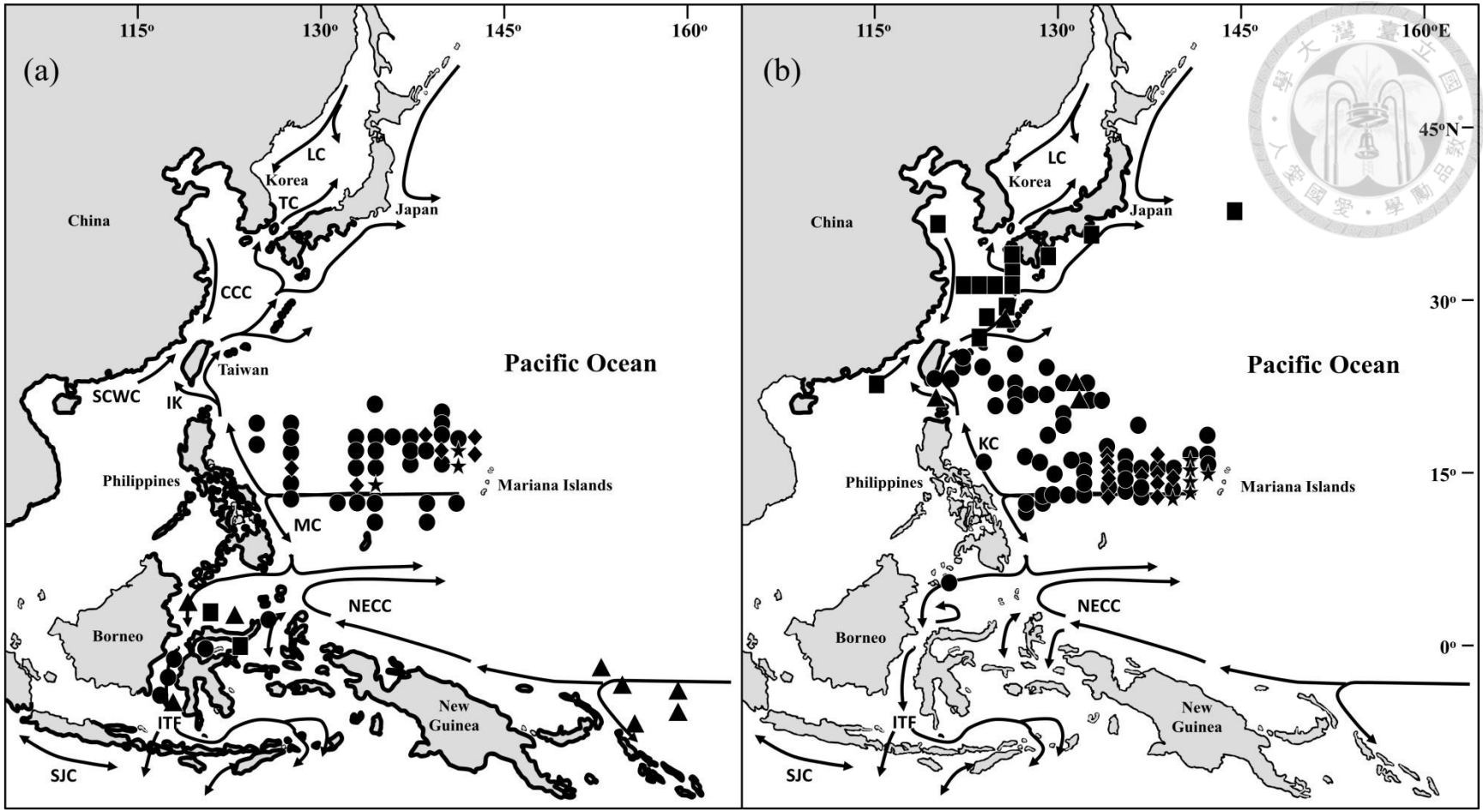


Figure 5. Map showing the species range (thick black lines along the coasts) of *Anguilla marmorata* (a) and *A. japonica* (b) in East Asia and the geographic distribution of their larvae (data from Liao et al. 1999, Kuroki et al. 2006 and Tsukamoto 2009). Preleptocephali (≤ 7 mm TL) (★), small leptocephali (≤ 10 mm TL) (♦), leptocephali (>10 mm TL) (●), metamorphosing leptocephali (▲) and oceanic glass eels (■). The detailed patterns of the general currents in the western North Pacific and central Indonesian Seas were also shown (adapted from Nitani 1972 and Lukas et al. 1991). NEC: North Equatorial Current, KC: Kuroshio Current, OC: Oyashio Current, TS: Tsushima Current, CCC: China Coastal Current, SCSWC: South China Sea Warm Current, IK: Intruded Kuroshio, MC: Mindanao Current, NECC: North Equatorial Counter Current.

1.5 Application of otolith daily growth increment and microchemistry to life history study

For many years, the life history events of anguillid eels have been the focus of a variety of researches. However such information about the early life histories of the anguillid eels have been slow to accumulate primarily because of the difficulty in collecting and keeping them alive due to their size and fragile body structure. Because of these constraints, otolith microstructure analysis has emerged as a useful and powerful technique in elucidating the early life history of the anguillid eels. Otoliths are small, paired calcified structures that aids in hearing and in the maintenance of equilibrium of all teleost fishes and are located in the fluid-filled chambers of the inner ear (Fig. 6). Fish otoliths are composed of calcium carbonate and an organic matrix and are both deposited in daily and annual increments as the fish grows, allowing the ages of the fishes to be determined on a daily and annual basis (Panella 1980). Recent chemical analytical techniques have enabled the identification of the life-history events in a variety of fish species by detecting major (>100 ppm), minor ($1 \leq$ ppm <100) and trace (<1 ppm) elements in the microstructures of their otoliths (Campana 1999; Arai 2002). This is because the depositions of the various elements in the growth increments of the otolith represent a permanent record of the environmental conditions experienced by the fish at a particular time (Campana et al. 2000). As a result of these advances, the growth increments in the otoliths have been widely used to study the different life history events in various eel species. It has been proven that the examination of the otolith microstructures and microchemistry is a useful tool in estimating various events in the life of the eel such as spawning and hatching dates, growth rate and life stage and habitat transitions. Recent progress in otolith analytical techniques have revealed considerable details of the early life history of various anguillid eels species including information on age, growth patterns, timing of metamorphosis of leptocephali, larval duration, recruitment to coastal waters, timing of

inshore migration of glass eels and others (Arai et al. 1997, 1999a,b,c, 2001a,b; Wang and Tzeng 1998; Ishikawa et al. 2001; Marui et al. 2001; Kuroki et al. 2008; Shiao et al. 2002). It was found that the growth increments in the otoliths of the newly hatched larvae and elvers of both temperate and tropical eel species are deposited on a daily basis thus it is possible to determine their various early life history details such as hatching date, age at first feeding, duration of leptocephalus stage, age at metamorphosis, oceanic glass eel stage and age at recruitment (Tsukamoto 1989; Martin 1995; Arai et al. 2000a; Cieri and McCleave 2001; Sugeha et al. 2001, Kuroki et al. 2006; Budimawan and Lecomte-Finiger 2007; Reveillac et al. 2009; Leander et al. 2013). It is also possible to calculate the growth rate by measuring the otolith growth increments. In addition, it was confirmed by various studies that there is a relationship between otolith microstructure and microchemistry (Otake et al. 1994; Arai et al. 1997; Arai et al. 2000b). A marked increase in otolith increment width coincides with a drop in otolith Sr: Ca ratios indicates the onset of metamorphosis, a common phenomenon in all anguillid eels (Otake et al. 1994; Arai et al. 1999a,b,c; Marui et al. 2001). Otake et al. (1994) suggested that this marked drop of otolith Sr: Ca ratios is related to the internal physiological changes that occurs during metamorphosis. Furthermore, Arai et al. (1997) reported that metamorphosis was completed before the increment width reached the maximum peak. These changes in otolith microstructure and microchemistry allowed us to have an insight on the timing and duration of the metamorphic period of the different anguillid eel species.

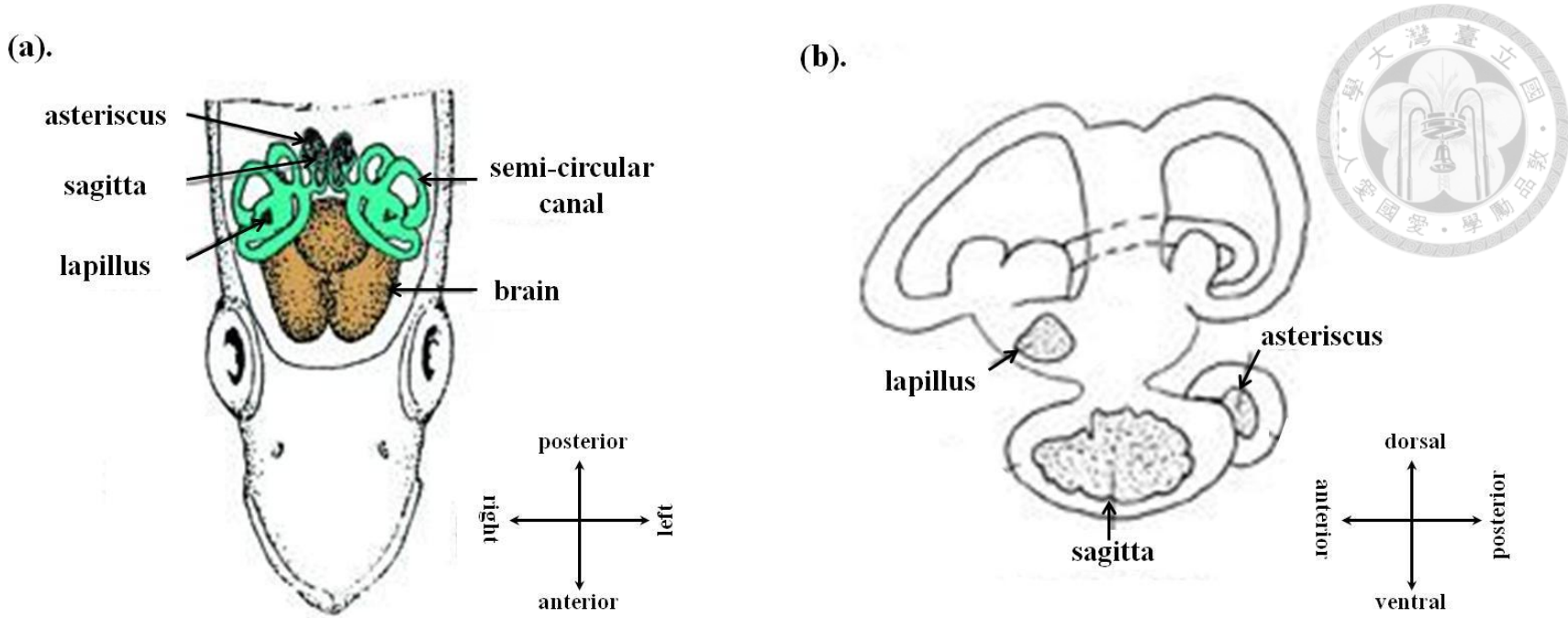


Fig. 6. The orientation of the semicircular canals and otoliths within the inner ear of teleost fish (a) and the transverse section of the inner ear showing the positions of each otoliths (b) (image modified from Panfili et al. 2002).

1.6 Current research status of *Anguilla marmorata*

Several aspects of the life history and ecology of the elvers of the temperate eel *A. japonica* in the estuaries around Taiwan has been well documented, including reports on metamorphosis and estuarine arrival (Tzeng 1990; Cheng and Tzeng 1996), timing of estuarine recruitment in relation to environmental conditions (Tzeng 1984a, 1985; Chen et al. 1994), fishing exploitation rates (Tzeng 1984b), effects of surface seawater temperature on the recruitment of elvers (Tzeng 2006), genetic composition of the recruiting glass eels (Chang et al. 2007) and the possible effects of environmental variations such as sunspot cycles and El Nino events on the glass eel catch (Han et al. 2009) and others. However, little is known concerning the tropical eel species on the eastern coast of Taiwan as previous studies were focused on the *A. japonica* populations on the southern, western and northern coast of Taiwan where their elvers were regularly collected for eel aquaculture. Accordingly, as compared with the information on *A. japonica*, relatively little is known about the life history of *A. marmorata*. It also did not help that *A. marmorata* was declared as an endangered species in Taiwan. Because of this ban, little information has been known about its population in Taiwan because its fishing and aquaculture is illegal before April 2009.

Not only its life history during the continental growth phase but also its marine larval life stage from the spawning ground to the estuary is still not well understood for *A. marmorata* as compared with that of the temperate eel species (McCleave 1993; Cheng and Tzeng 1996; Wang and Tzeng 2000; Tzeng 2003; Edeline et al. 2009; Miller 2009). Although recent research efforts have resulted in a basic understanding of the ecology of the anguillid eels, early life history details such as metamorphosis from leptocephalus to glass eel stage as well as from glass eel to elvers stage remains to be understood. Similarly, in the Philippines, the first country the drifting

leptocephalus encountered as they migrate and colonize continental East Asia, information such as population structure, recruitment dynamics, species composition and abundance, etc. is still lacking. Such information is essential for the sustainable fishery and resource conservation of the tropical anguillid eel populations. It is because of this need for information regarding the life history of *A. marmorata* that this present study was conceived.

Also, to understand the decline in glass eel recruitment, it is necessary to study the early life history of anguillid eels. The life history of eels during the continental growth phase is well documented, but knowledge of the marine larval life stage from the spawning ground to the estuary is still fragmented (McCleave 1993, Cheng and Tzeng 1996, Wang and Tzeng 2000, Tzeng 2003, Edeline et al. 2009, Miller 2009). Early life-history information is very important because it is a key factor in understanding possible reasons for recruitment success or failure of anguillid eels and also for their artificial propagation.

1.7 Objectives of this study

The present study aims to examine the life history of the tropical eel *A. marmorata* in northwestern Pacific from marine larval phase up to continental growth phase by evaluating its recruitment pattern in the estuary and by analyzing its otolith microchemistry and microstructures. In addition, its life history and evolution in the northwestern Pacific were compared with that of *A. japonica*. Specifically this study aimed to:

- Examine the early oceanic growth phase of *A. marmorata* using information such as the timing of metamorphosis from leptocephalus to glass eel, inshore migration period of the glass eel, age and size at estuarine arrival and the growth rate of the leptocephalus and elver.
- Determine the possible mechanism for the segregative migration and recruitment of *A. marmorata* and *A. japonica* in the northwestern Pacific.
- Assess the recruitment pattern of *A. marmorata* and other anguillid eel species in Hsiukuluan River, Eastern Taiwan from 2005-2009 to clarify their seasonality and abundance.
- Determine the migratory environmental history and habitat use of adult *A. marmorata* in eastern Luzon, Philippines.

2. MATERIALS AND METHODS

2.1 Glass eel collection

2.1.1 Temporal sampling

To determine the species composition, seasonal occurrence and recruitment pattern of anguillid eel in eastern Taiwan, glass eel were sampled monthly in 2005 and 2007-2009 at 2 stations in the lower reach of the Hsiukuluan River (Fig. 7). Hsiukuluan River is the largest river in eastern Taiwan with a length of 81 km and a drainage area of 1790 km^2 (Shiao et al. 2003). The 1st station was located in the river mouth, and a traditional triangle net was used for glass eel collection; the 2nd station was located 2 km upstream where a fish way trap was set up in an artificially dug water channel that was about 20 m long, 1.5 m wide, and 25 cm deep (Fig. 8). After collection, specimens were immediately preserved in 75% ethanol. Environmental parameters such as dissolved oxygen (DO), salinity (conductivity), pH, water temperate, water velocity, and turbidity were also recorded at every sample collection.

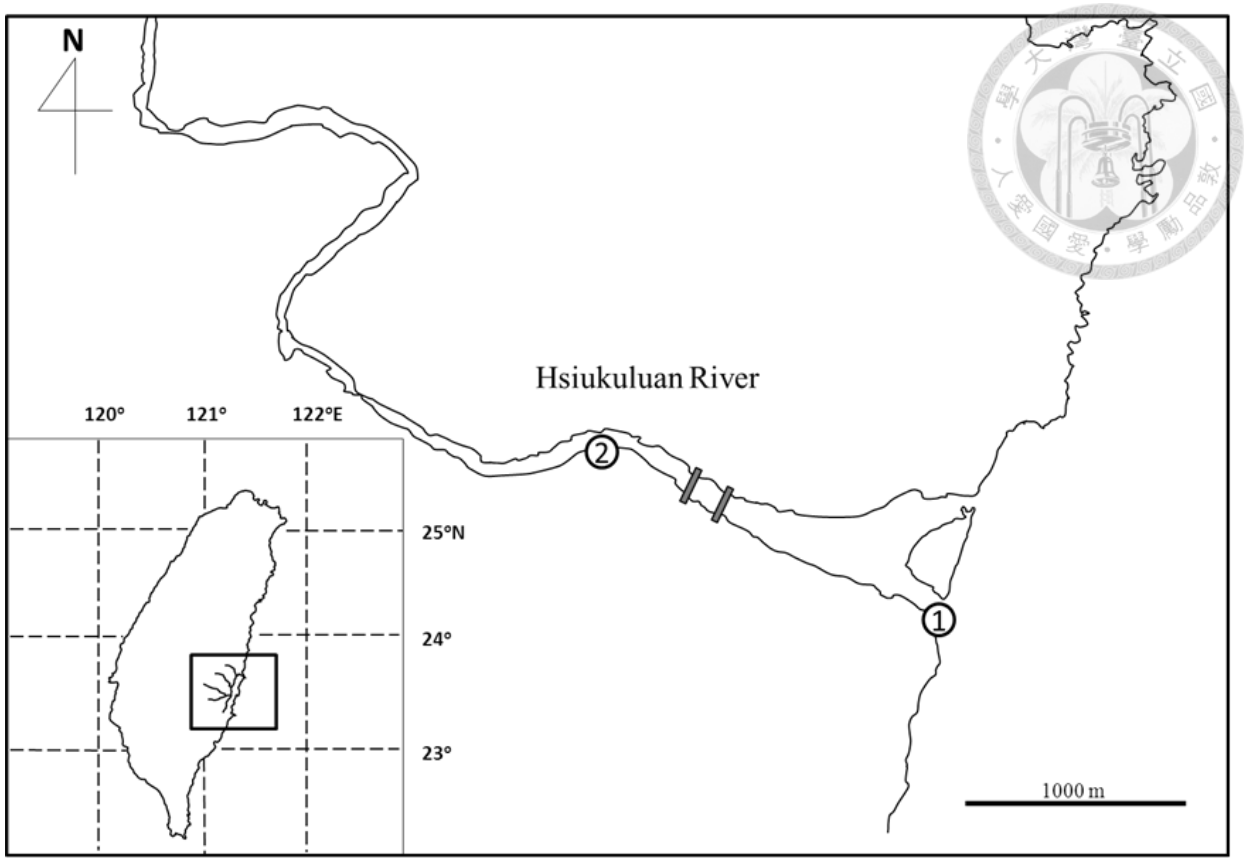


Fig 7. Map showing glass eel sampling stations (1 and 2) in the lower reach of the Hsiukuluan River in eastern Taiwan. The bars indicate the Juisiu and Long Rainbow Bridges.

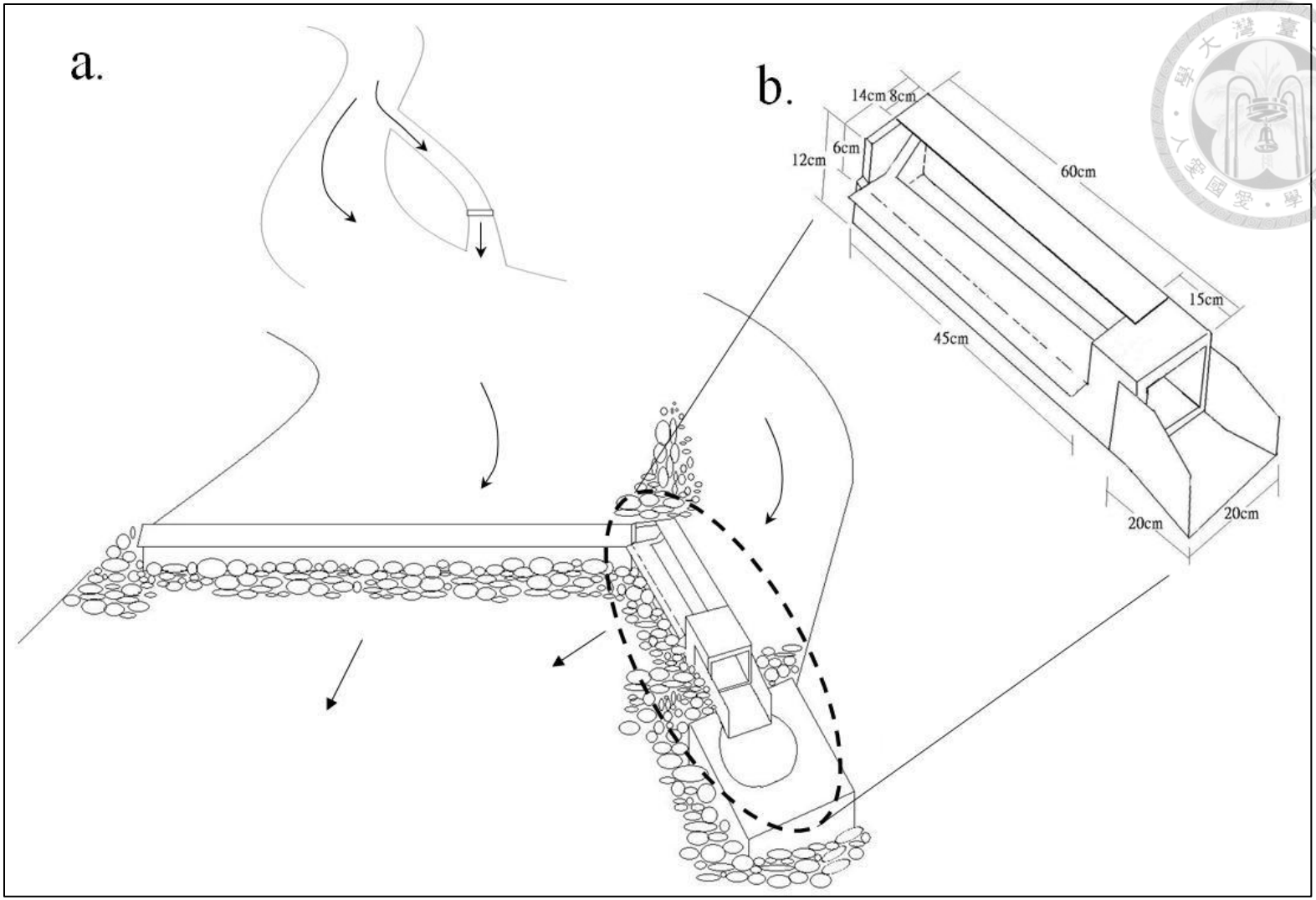


Fig. 8. Fish way trap used to collect the migrating fish larvae in the lower reach of Hsiukuluan River (a) and the dimension of the tin trap (b). Arrows represents the direction of the river flow. Figure provided by Dr. R.T. Chen.

Table 1. Sampling information and sample sizes of the anguillid glass eel collected in the lower reach of Hsiukuluan River, eastern Taiwan.

Sampling period		Sample size		
		Stn. 1	Stn. 2	Total
2005	June	0	151	151
2007	July	7	19	26
	Sept.	0	8	8
2008	Apr.	0	1	1
	May	0	48	48
	June	31	23	54
	July	0	13	13
	Aug.	56	0	56
	Sept.	4	1	5
	Oct.	36	0	36
	Nov.	280	0	280
	Dec.	62	0	62
2009	Jan.	1	0	1
	Feb.	1	0	1
	Mar.	42	2	44
	Apr.	64	0	64
	May	17	0	17
	June	61	0	61
	July	69	0	69
	Aug.	4	0	4
	Sept.	1	2	3
Total		736	268	1004

2.1.2 Spatial sampling

A total of 168 *A. marmorata* glass eels were collected from the estuaries of the Hsiukuluan River, eastern Taiwan (*n* = 86) on 20 May 2008, the Cagayan River, northern Philippines (*n* = 45) on 19 May 2008, and the Kurio River, southern Japan (*n* = 37) on 6 June 1996 (Fig. 9) to determine the possible effects of early life history traits on its latitudinal distribution in the northwestern Pacific together with *A. japonica*. All specimens were immediately preserved in 95% ethanol after measuring the total lengths (TLs) to the nearest millimeter (mm). On the other hand, *A. japonica* specimens examined from a previous study (Cheng and Tzeng 1996) were collected from estuaries of the Tungkang River, southern Taiwan (*n* = 60) on 30 December 1992 and 24 March 1993, the Shuangshi River, northern Taiwan (*n* = 60) on 20 December 1992 and 17 February 1993, the Mingchiang River, eastern China (*n* = 30) on 1 March 1993, the Chyantarng River, eastern China (*n* = 30) on 17 February 1993, the Yalu River, northern China (*n* = 30) on 3 May 1993, and the Ichinomiya River, eastern Japan (*n* = 30) on 10 January 1994 (Fig. 9).

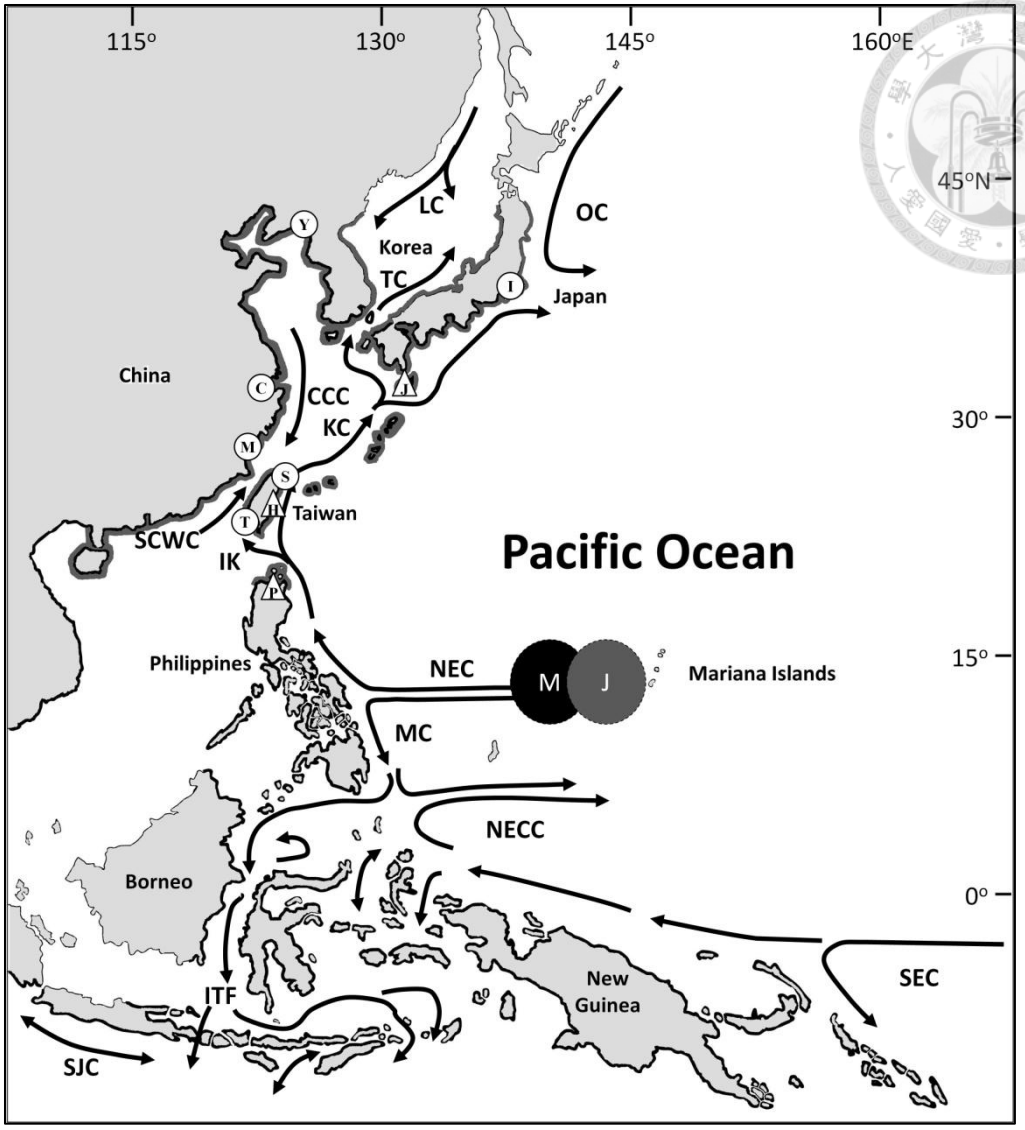


Fig. 9. Map showing the geographic distributions of *Anguilla japonica* (thick gray lines on the coastlines) and *A. marmorata* (thick black lines on the coastlines) in East Asia and collection sites of samples analyzed in this study (Δ, *A. marmorata*; O, *A. japonica* ; Cheng and Tzeng 1996). General patterns of current systems in the western North Pacific and central Indonesian Seas (adapted from Nitani 1972, Lukas et al. 1991, and Revendin et al. 1994) and spawning grounds of *A. japonica* (gray circle with the letter J) and *A. marmorata* (black circle with the letter M) (Kuroki et al. 2009, Tsukamoto et al. 2011) are also shown. Sampling locations: Tungkang River (T) and Shuangshi River (S), Taiwan; Mingchiang River (M), Chyantarng River (C), and Yalu River (Y), China; Ichinomiya River (I), Japan; Cagayan River (P), the Philippines; Hsiukuluan River (H), Taiwan; and Kurio River (J), Japan. NEC, North Equatorial Current; KC, Kuroshio Current; OC, Oyashio Current; TS, Tsushima Current; CCC, China Coastal Current; SCSWC, South China Sea Warm Current; IK, Intruded Kuroshio; MC, Mindanao Current; NECC, North Equatorial Counter Current; SEC, South Equatorial Current.

Table 2. Sampling information and sample sizes of *A. japonica* and *A. marmorata* glass eel collection in various rivers and estuaries in East Asia. Values inside the parentheses indicate the number of individuals used for otolith analyses.

Species	Sampling site	Sampling date	*n*
*A. japonica**	Tungkang River, Taiwan	30 Dec. 1992	30 (16)
		24 Mar. 1993	30 (14)
	Shuangshi River, Taiwan	20 Dec. 1992	30 (12)
		17 Feb. 1993	30 (13)
	Mingchiang River, China	1 Mar. 1993	30 (20)
	Chyantarng River, China	17 Feb. 1993	30 (23)
	Yalu River, China	03 May 1993	30 (23)
	Ichinomiya River, Japan	10 Jan. 1994	30 (10)
			240 (131)
A. marmorata	Cagayan River, Philippines	19 May 2008	45 (13)
	Hsiukuluan River, Taiwan	20 May 2008	86 (13)
	Kurio River, Japan	6 Jun. 1996	37 (15)
			168 (41)

*from Cheng and Tzeng (1996)

2.2 Juvenile eel collection

Juvenile *A. marmorata* were collected in Aguang River in eastern Luzon, Philippines (Fig. 10) from June-August 2012 to determine the migration pattern and habitat use. The eels were caught using an electro-fishing gear with the help of some local fishermen in the area (Fig. 11). For comparison, samples from an aquaculture farm in Sanya, southern Hainan province, China was included in the analyses.

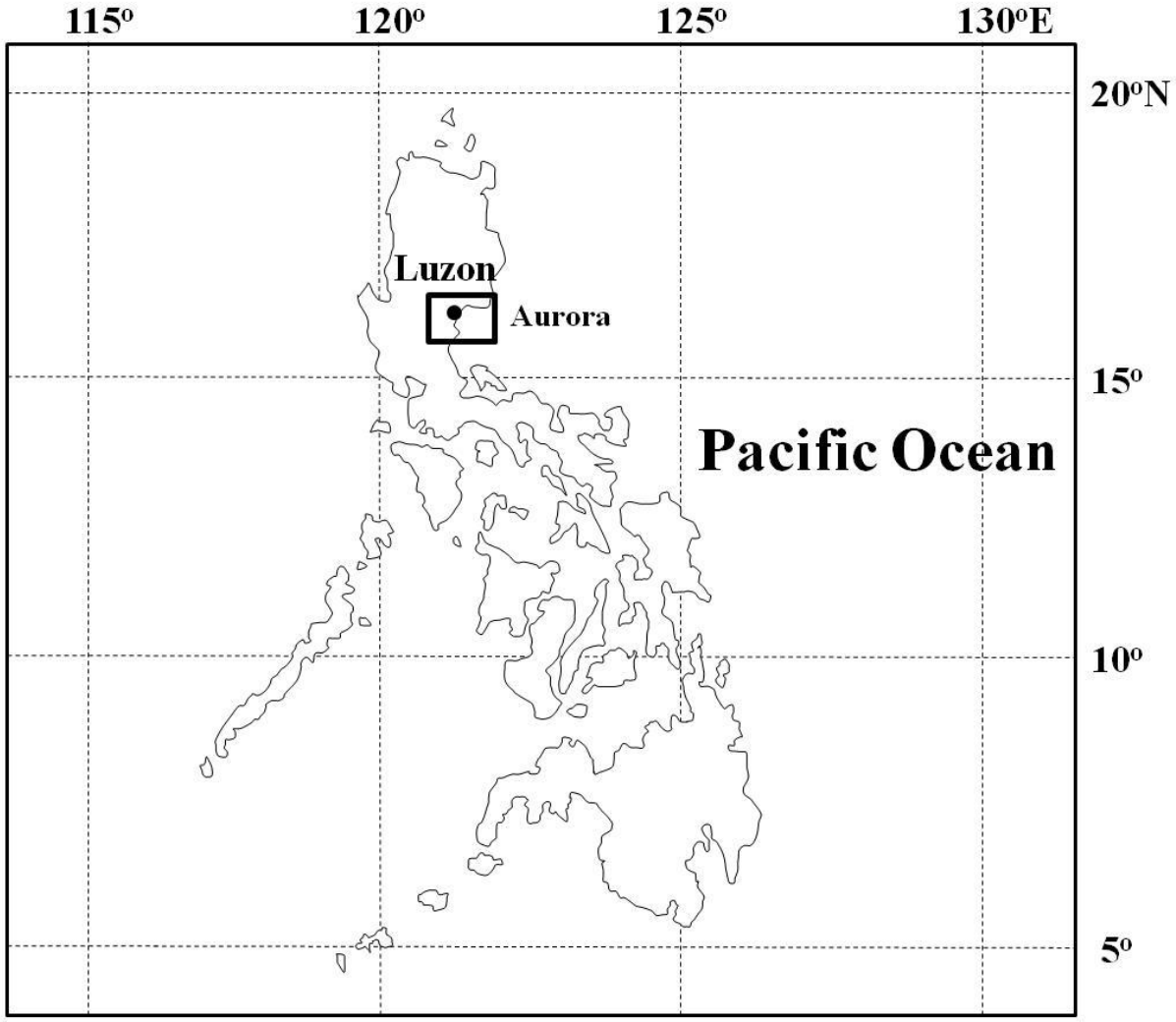


Fig. 10. Sampling location of the adult eels in a river system in Aurora province in eastern Luzon, Philippines.

Fig. 11. Electro-fishing gear (a) used to catch *A. marmorata* juveniles in a river system (b) in Aurora province, eastern Luzon, Philippines.

2.3 Glass eel species identification and morphometric measurements

Glass eel species were identified using morphological characters and pigmentation patterns summarized in Fig. 12 which was modified from Tzeng (1982, 1983a) and Tzeng and Tabeta (1983). Morphological characters, including total length, pre-dorsal fin length (PDL), pre-anal fin length (PAL), and ADL, were measured to the nearest 0.1 mm (Fig. 13). Total length (TL) was determined by measuring the distance between the tip of the snout and the end of the tail, while the PDL was determined by measuring the distance from the tip of the snout to the origin of the dorsal fin. The PAL was determined by measuring the distance from the tip of the snout to the origin of the anal fin. The ADL, on the other hand, was the difference in distance between the origin of the dorsal and anal fins in percent of the total length (ADL/%TL). In addition, tail bud and caudal fin cutaneous pigmentation patterns, which appear during the glass eel pigmentation process, were also used for species identification.

Glass eels were classified into long- and short-finned types according to the value of ADL/%TL. Individuals with ADL/%TL values of < 5% were classified as short-finned eels (the pre-dorsal fin origin is closer to the anus than jaw), while individuals with AD/%TL values of > 5% were classified as long-finned eels (the pre-dorsal fin origin is closer to the jaw than anus) (Ege 1939, Tesch 2003). *Anguilla bicolor pacifica* is a short-finned eel with pigmentation in the tail bud that extends to the caudal fin, while the rest are long-finned eels. The 3 long-finned eel species were then separated according to the cutaneous pigmentation patterns on the posterior part of the body. *Anguilla japonica* has no pigmentation at this stage, while both *A. marmorata* and *A. luzonensis* and/or *A. celebesensis* have more or less the same pigmentation patterns in the tail bud. *Anguilla marmorata* and *A. luzonensis* and/or *A. celebesensis* were then separated according to ADL/%TL values. The ADL/%TL was reported to differ between these species but

there is some degree of overlap (Tzeng 1982, Teng et al. 2009, Watanabe et al. 2009). Individuals with ADL/%TL values of > 13 were classified as *A. marmorata*, while those with values of < 13 were classified as *A. luzonensis* and/or *A. celebesensis*. Furthermore, the reliability of using ADL/%TL to discriminate *A. marmorata*, *A. luzonensis*, and/or *A. celebesensis* was tested by molecular identification. In total, 6 individuals with minimum, medium, and maximum values of ADL/%TL were chosen for the DNA analysis.

The morphometric datasets were subjected to normality and equal variance tests because of the unequal sample sizes. If the dataset passed the test, significant differences were examined using a one-way analysis of variance (ANOVA) followed by pairwise multiple comparisons using the Holm-Sidak method. On the other hand, if the dataset failed the test, significant differences were examined using a Kruskal-Wallis ANOVA on ranks followed by multiple comparisons using Dunn's method. Holm-Sidak and Dunn's post-hoc tests were conducted to detect pairwise differences between species with an overall alpha level of 0.05. All statistical analyses were carried out using SigmaStat software vers. 3.5 (Systat Software, San Jose, CA, USA).

2.4 DNA extraction, polymerase chain reaction (PCR) amplification and phylogenetic analysis

Total genomic DNA was extracted from muscle tissues of individuals with minimum, medium, and maximum values of ADL/%TL using a DNA purification and extraction kit. A pair of oligonucleotide primers, H15341 (5'-TGCTAACGATGCCCTAGTGG-3') and L151341 (5'-CTAGTCAACCTACTAATGGG-3') was used to amplify a fragment of Cyt *b* using PCR amplification (Han et al. 2002). PCR amplification was carried out in a 25-µl reaction mixture containing 0.5 µl template DNA, 2.5 µl 10x reaction buffer, 0.5 µl dNTP, 1 µl of each forward

and reverse oligonucleotide primers, 0.25 µl DNA Taq polymerase, and 19.25 µl double-distilled water. The thermal profile consisted of initial denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50-55°C for 1 min, and extension at 72°C for 30 s, with a final extension at 72°C for 10 min. PCR products were electrophoresed on a 1% agarose gel and stained with ethidium bromide (EtBr) for band characterization via ultraviolet trans-illumination. Sequencing reactions were performed using an ABI PRISM 377 Auto DNA Sequencer (Applied Biosystems, Foster City, CA, USA).

The generated sequences were compared to the mitochondrial (mt)DNA Cyt *b* sequences of all known species and subspecies of *Anguilla* retrieved from GenBank (accession nos.: AB038556, AB469437, and AP007233-49) to determine their phylogenetic relationships using the Neighbor-joining (NJ) method with the Kimura two-parameter model as implemented in MEGA 4.1 (Tamura et al. 2007). The resultant topology was assessed by bootstrapping with 1000 replications.

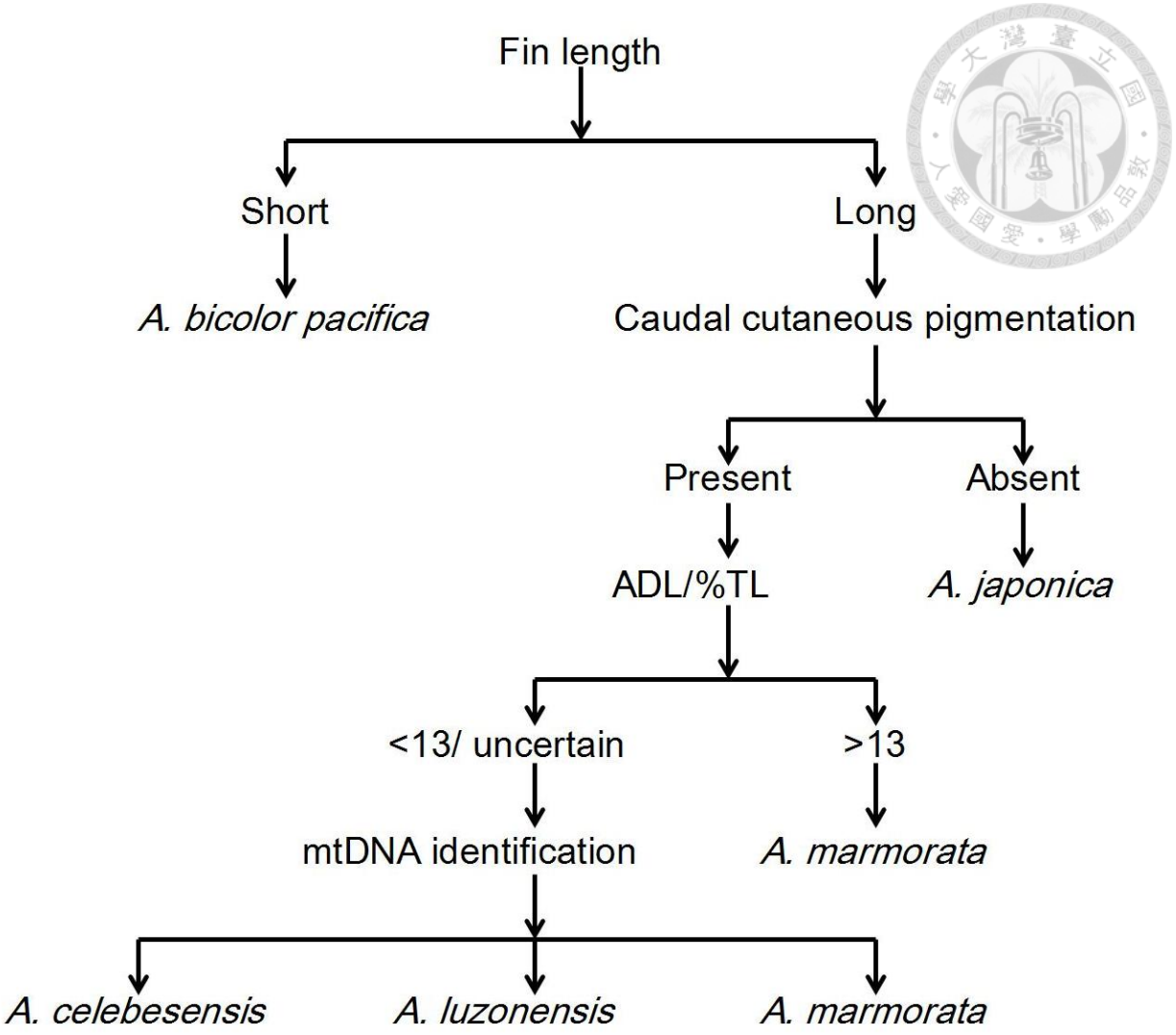


Fig. 12. Schematic diagram of the methods used for anguillid glass eel species identification.

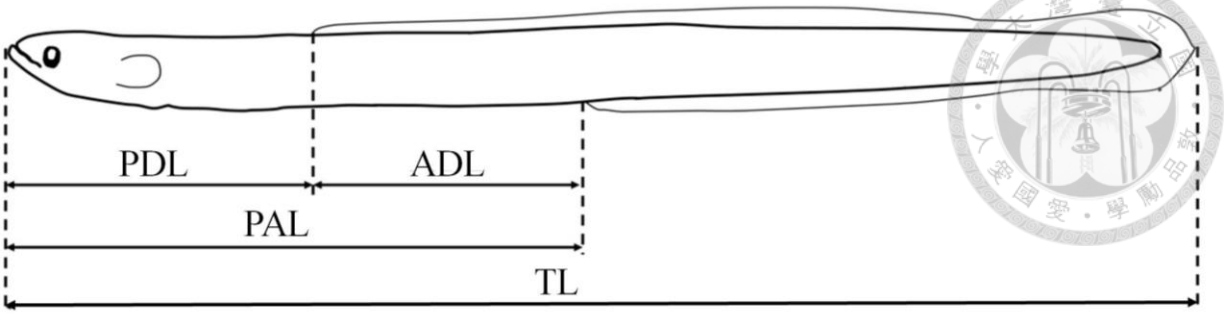


Fig. 13. Diagram showing morphometric measurements of the glass eel. PD, pre-dorsal length; AD, ano-dorsal length, PA, pre-anal length and TL, total length. Glass eels were classified into long- and short-finned types according to the value of ADL/%TL.

2.5 Determination of glass eel developmental stages

Developmental stages from glass eel to elver were also determined according to the extent (or absence) of skin pigmentation over the head, tail, and other body regions following methods described by Strubberg (1913), Bertin (1956), and Tesch (1977, 2003) (Fig. 14a-b, Table 1). Post-metamorphic juveniles were sub-classified into stages V_A, V_B, VI_{A1}, VI_{A2}, VI_{A3}, VI_{A4}, VI_B, and VII. Juveniles up to stage VI_{A2} were classified as glass eels, while those in stages VI_{A3} and VI_{A4} were in the transition stage to elvers, which become fully pigmented at stage VI_B stage (Fukuda 2010). Stage VI_B indicates the end of pigmentation, while stage VII represents the fully pigmented, benthic elver (Tabeta and Mochioka 2003).

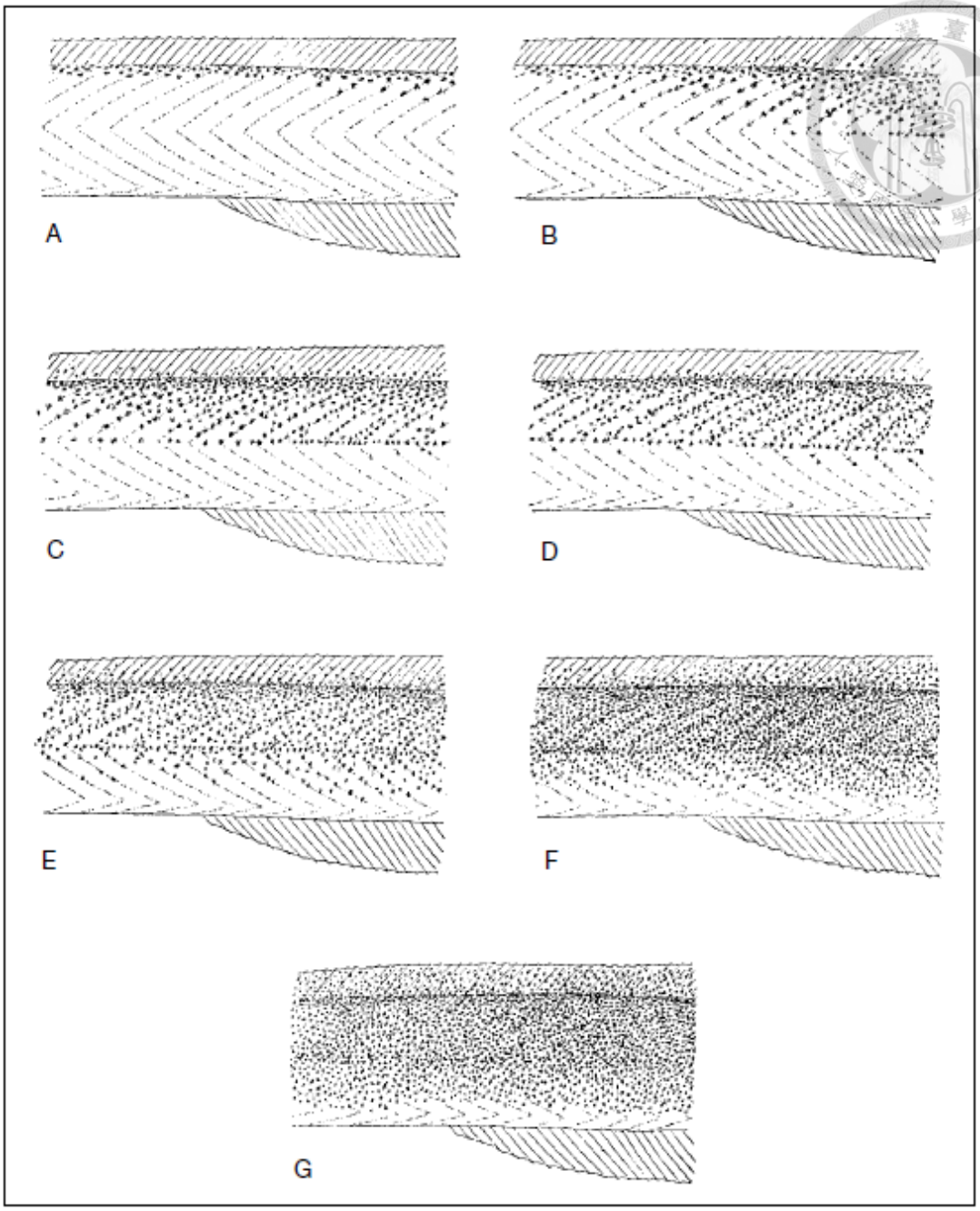


Fig 14a. Progressive sub-epidermal pigmentation in the anal region of the glass eel used to determine the different ontogenetic stages (after Strubberg, 1913; Bertin 1956; Tesch 2003). A: Stage VIA_1; B:Stage VIA_2; C: Stage VIA_3; D,E,F: Stage VIA_4; G: Stage VIB.

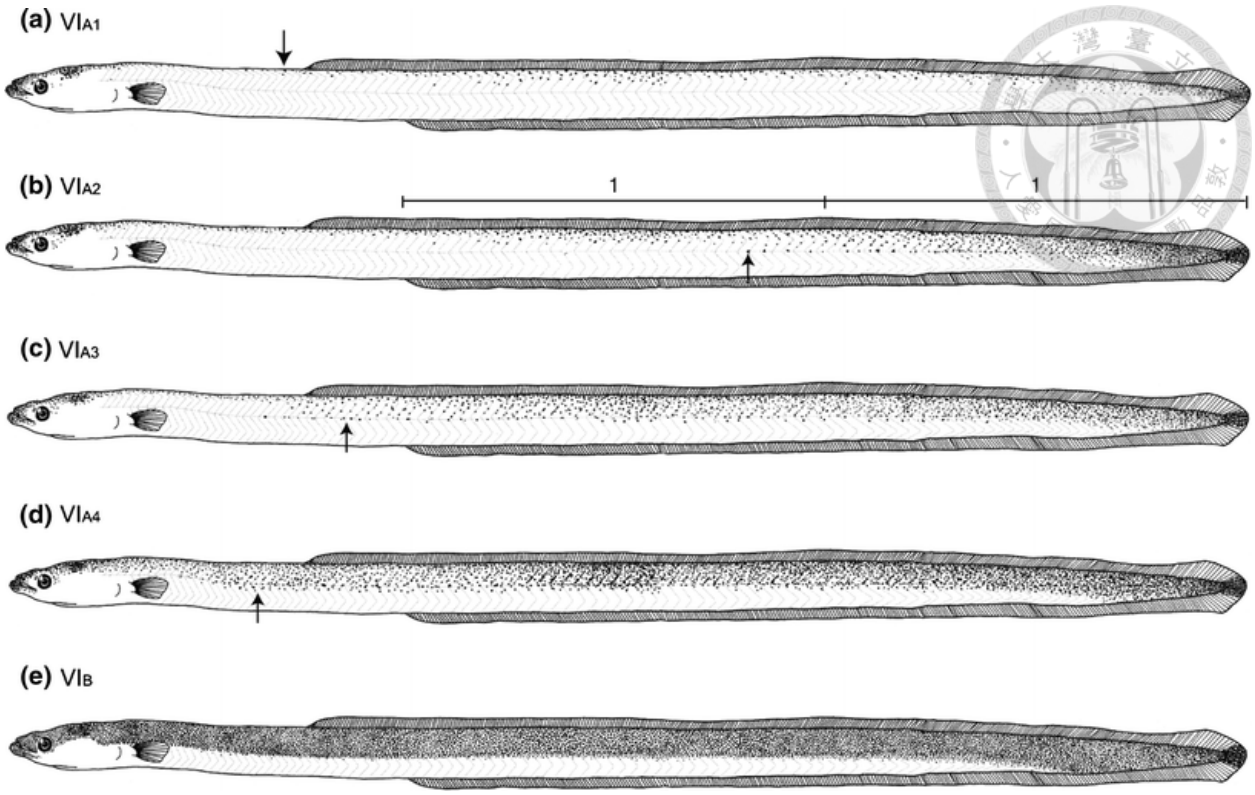


Fig. 14b. Developmental stages (VI_{A1} to VI_B) in Japanese eels showing pigmentation process from glass eel to the fully pigmented elver stage at VIb. Arrows indicates the location of the unique pigmentation characteristic of each stage (adapted from Fukuda et al. 2013)

Table 3. Development of pigmentation in anguillid eels (Tesch 2003).

Stage	Characteristics
I	Larva, fully grown leptocephalus
II	Semilarva, pigmentation on the posterior end of the spinal chord
III	Semilarva, pigmentation on the nerve chord becomes more extensive, skin pigment also seen at the tip of the caudal fin
IV	Semilarva, pigmentation on the nerve chord reaches the head
V_A	Metamorphosis complete, eel-like form, no external pigment (glass eel) except the caudal spot
V_B	No pigment on the back, body or tail region, except for the skull, caudal spot and some rostral pigment
VI_{A1}	Development of pigmentation along the whole dorsum, post-anal dorsolateral pigment develops, post anal, no clear mediolateral melanophores (Fig.14b-a)
VI_{A2}	No pre-anal ventrolateral pigment. Post-anal development mediolateral pigment (Fig. 14b-b)
VI_{A3}	No pre-anal ventrolateral pigment. Clear pre-anal development of mediolateral pigment, post-anally over almost entire dorsum, pigment rows along the myosepta and in places doubling of the mediolateral melanophores (Fig. 14b-c)
VI_{A4}	Clear development of pre-anal ventrolateral pigmentation. Initially, in places, a doubling of the mediolateral melanophores in the pre-anal region (Fig. 14b-d), post-anal pigment between the myosepta in the ventral region (Fig. 9b-e) and finally, similar changes in the pre-anal region (Fig. 14b-f)
VI_B	Pigment rows along the myosepta becoming indistinct. Lateral line still recognizable, as are the individual melanophores on the head, cheek, behind and below the eyes and on the lower jaw (Fig. 14b-g)

2.6 Morphometric measurements of adult eels

Morphological characters, including total length, pre-dorsal fin length (PDL), pre-anal fin length (PAL), and ADL, were measured to the nearest 0.1 mm (Fig. 15). Total length (TL) was determined by measuring the distance between the tip of the snout and the end of the tail, while the PDL was determined by measuring the distance from the tip of the snout to the origin of the dorsal fin. The PAL was determined by measuring the distance from the tip of the snout to the origin of the anal fin. The ADL, on the other hand, was the difference in distance between the origin of the dorsal and anal fins in percent of the total length (ADL/%TL).

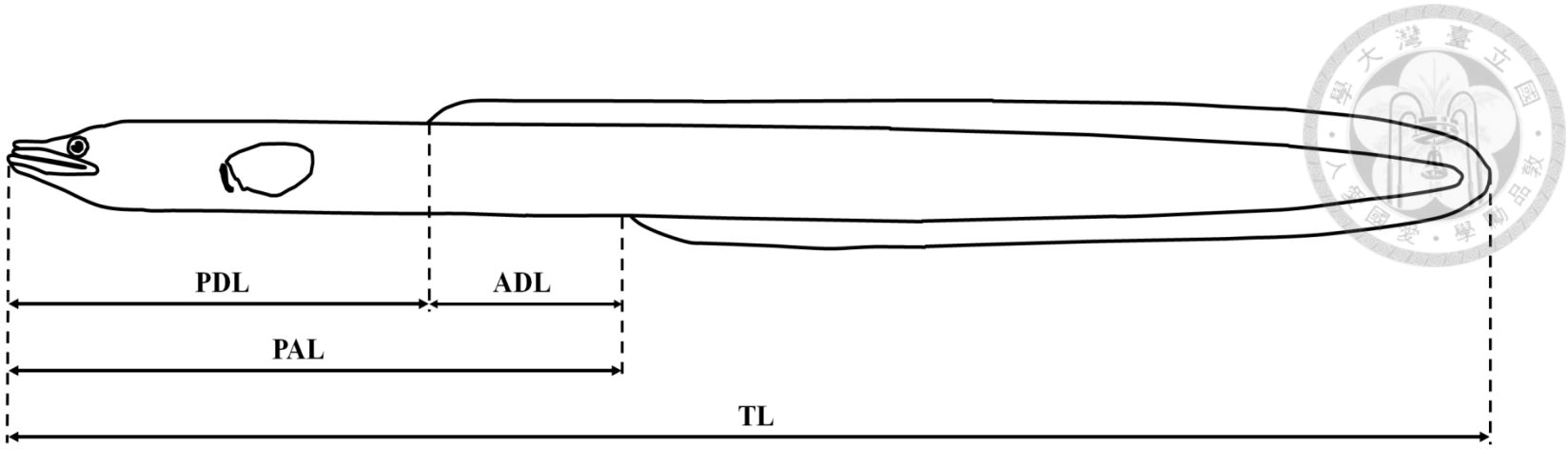
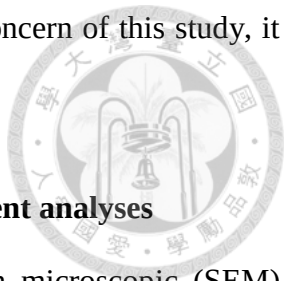


Fig. 15. Diagram showing morphometric measurements of the adult eel. PDL, pre-dorsal length; ADL, ano-dorsal length, PAL, pre-anal length, SL, standard length, and TL, total length.

2.7 Otolith extraction and preparation for daily growth increment and microchemistry analyses

Sagittal otoliths, the largest of the 3 pairs of otoliths in the inner ear, were extracted and embedded in epofix resin. The embedded otolith was ground and polished with a grinding machine until the primordium was exposed. Sr and Ca concentrations were measured from the primordium to the otolith edge at 10-μm intervals with an electron beam of 5 x 4 μm in diameter, using an electron probe microanalyzer equipped with a wavelength-dispersive spectrometer (WDS-EPMA; JEOL JXA-8900R, Tokyo, Japan). The accelerating voltage was set to 15 kV and the probe current to 3 nA. The peak concentration of Sr was counted for 80 s with background measurements for 20 s on each side. On the other hand, the peak concentration of Ca was counted for 20 s and each background for 10 s. Strontianite ($SrCO_3$: USNM-R10065) and calcite ($CaCo_3$: USNM-36321) from the Department of Mineral Sciences, National Museum of Natural History, Smithsonian Institution, Washington, DC, were used as standards to calibrate the Sr and Ca concentration in eel otoliths. After the microchemical analysis, the otolith was polished to remove the carbon layer, and etched for 1~2 min with 5% ethylenediaminetetraacetic acid (EDTA) to reveal the DGIs (annual rings for adult otoliths). Procedures for embedding, sectioning, polishing, and etching otoliths to reveal the DGIs and measuring the otolith Sr:Ca ratios followed those described in previous studies by Tzeng (1990 1996), while the procedure for measuring the otolith Sr:Ca ratios followed that of Tzeng and Tsai (1994). On the other hand, the ages of the adult eel samples were determined by counting the annuli that are deposited on a yearly basis in their otoliths. The criteria used to interpret the otolith annuli of *A. marmorata* followed that used in the temperate eels. However, the determination and interpretation of annuli in tropical eel species is sometimes very difficult and unreliable due to the existence of false

annuli. Although the ages of the adult *A. marmorata* were not the main concern of this study, it was still estimated.



2.8 Life history parameters obtained from otolith daily growth increment analyses

The DGIs in the otoliths were examined from scanning electron microscopic (SEM) photographs at various magnifications (200x, 1000x, and 1500x). Both the DGIs and Sr:Ca ratios were measured along the longest otolith axis. Growth checks of each early life-history event or transition recorded in the otoliths were identified using both otolith microstructures (the DGI width) and microchemistry (Sr:Ca ratios). DGIs in each of the developmental stages and otolith radius were counted and measured from these landmarks as shown in Fig. 16. Because otolith increments in *A. marmorata* and *A. japonica* were confirmed to be deposited on a daily basis (Tabeta et al. 1987, Umezawa et al. 1989, Sugeha et al. 2001b), the increment number was considered as the daily age in each individual examined in the present study. The drastic change in otolith Sr:Ca from the primordium to the otolith edge coincided with major life-history events in the life of the young eels, like 1st feeding, metamorphosis, etc., as reported in previous studies for both temperate and tropical anguillid species (Tzeng and Tsai 1992 1994, Otake et al. 1994 1997, Tzeng 1996a, Arai et al. 1997 1999a b c, Cieri and McCleave 2001, Marui et al. 2001). The age of the leptocephalus at the onset of metamorphosis (T_m) was determined from the number of DGIs between the primordium (P) and MC where the increment pattern and Sr:Ca ratios dramatically changed (Otake et al. 1994, Tzeng and Tsai 1994, Tzeng 1995, Kuroki et al. 2005, Arai et al. 2002a). To estimate the T_m, 13 d (adjustment factor, N_0) was added to the number of DGIs, because a previous study found that no increment was deposited in the core of the otolith during the yolk-sac stage, and otolith growth increment deposition only commences once a larva begins feeding 13 d after hatching (Tanaka et al. 1995). The duration of the

metamorphosis stage was determined by counting the number of DGIs between the onset of a marked increase in the otolith increment width and its maximum peak. The amount of time between metamorphosis and estuarine arrival ($T_{t\text{-}m}$) was calculated by counting the number of DGIs between the MC and the edge of the otolith, while the age at recruitment (T_t) was determined as the number of DGIs between the hatch check and otolith edge. On the other hand, radii from the primordium to the 1st feeding check (R_f), to the MC (R_m), and to the otolith edge (R_t), and the distance from the MC to the otolith edge ($R_{t\text{-}m}$) were measured along the longest sagittal axis of the otolith (Fig. 11). Otolith growth rates at different developmental stages were calculated by dividing the otolith radius by the DGI (equations 1-3). Because increments near the metamorphosis zone in the otolith of some samples were often diffusive and obscure, the daily age of samples without counting DGIs was calculated from both the otolith growth rate and otolith radius (equations 4-6) as described by Wang and Tzeng (2000):

(1) Overall growth rate of otolith, $$Gt = \frac{Rt}{Tt}$$

(2) Early growth rate of otolith, $$Gm = \frac{Rm}{Tm}$$

(3) Estuarine growth rate of otolith, $$Gt - m = \frac{Rt - m}{Tt - m}$$

(4) $$Tm = \frac{Rm - Rf}{Gm} + No$$

(5) $$Tt - m = \frac{Rt - m}{Gt - m}$$

$$(6)\ Tt = Tm + Tt - m$$

where G_m and $G_{t\text{-}m}$ were obtained from equations (2) and (3) and N_0 is the adjustment factor (5 days) for yolk-sac stage durations. The differences in total length, daily age and growth rate between *A. japonica* and *A. marmorata* were tested by analysis of variance (ANOVA) as implemented in Sigma-Stat v3.1 (Systat Software, Inc., San Jose, California, USA).

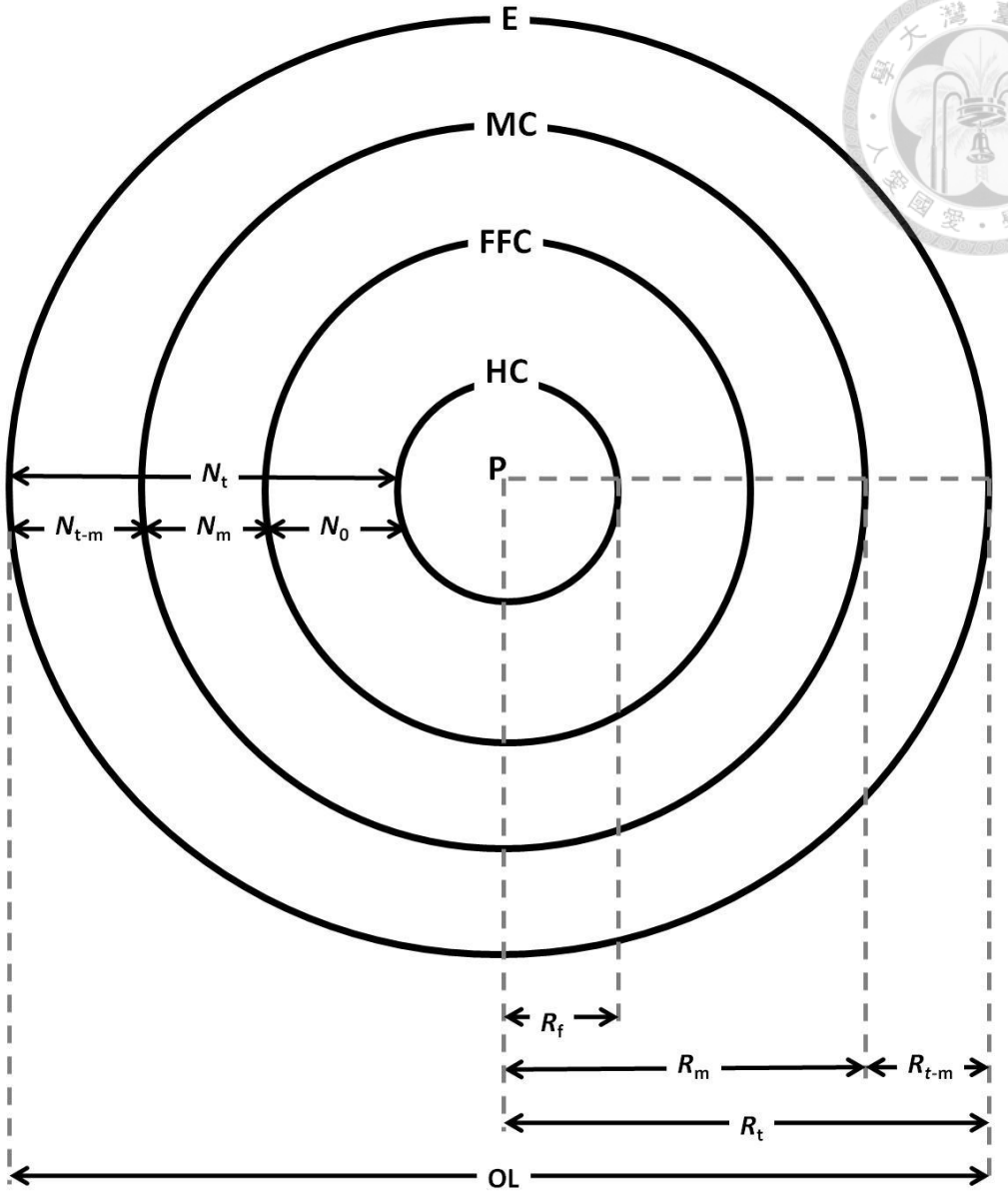


Fig. 16. Schematic diagram of the measurements of radii and counts of the daily growth increments in the otoliths of the elvers (modified from Cheng and Tzeng 1996; Wang and Tzeng 2000). P: primordium; HC: hatch check; FFC: first feeding check; MC: metamorphosis check; E: edge; R_f, R_m, R_t: radii from the primordium to the first feeding check, to the metamorphosis check and to the otolith edge, respectively; $R_{t\text{-}m}$: distance from the metamorphosis check to the otolith edge; OL: otolith length; T_m, T_t, $T_{t\text{-}m}$: counts of the daily growth increments on the radii of R_m, R_t and the section $R_{t\text{-}m}$ between MC and E, respectively.

3. RESULTS

3.1 Species identification for the glass eel recruiting to Hsiukuluan River, Eastern Taiwan

Species of the glass eel were preliminarily identified using the caudal pigmentation pattern and ADL/%TL values (Table 4; Fig. 17). *Anguilla japonica* (Fig. 17a) and *A. marmorata* (Fig. 17b) are longfin species while *A. bicolor pacifica* is a shortfin species (Fig. 17c). Cutaneous pigmentation patterns on the caudal part of glass eels differ among species and can be classified into 3 types (Fig. 18): type 1 lacks pigmentation on both the tail bud and caudal fin, i.e., *A. japonica* (Fig. 18a); type 2 has large patches of small (stellate) melanophores on the caudal fin, i.e., *A. bicolor pacifica* (Fig. 18b); and type 3 has a large patch of diffused melanophores on the tail bud, i.e., *A. marmorata, A. luzonensis*, and/or *A. celebesensis* (Fig. 18c). Pigmentation patterns alone cannot be used to distinguish these 3 pigmented tropical eel species so a subset (424 individuals) was chosen to discriminate the species. Individuals with ADL/%TL values of > 13 were classified as *A. marmorata* (A) while those with ADL/%TL values of < 13 were classified as *A. luzonensis* and/or *A. celebesensis* (uncertain or B) (Table 2). On the other hand, unidentified individuals were labeled as uncertain. Individuals with ADL/%TL values of > 13 totaled 358 individuals, while those with values of < 13 totaled only 27 individuals. Based on pigmentation patterns and morphometric analyses, very few *A. japonica* (5 individuals) or *A. bicolor pacifica* (13 individuals) specimens were identified. A subset of samples with ADL/%TL values of < 13 and of > 13 were chooses for DNA analyses to check the reliability of using ADL/%TL to discriminate *A. marmorata*, *A. luzonensis* and/or *A. celebesensis*. The phylogenetic analysis did not support the occurrence the new species and/or *A. celebesensis* in Taiwan based on species-specific differences in ADL/%TL values. Sequences of eel species with ADL/%TL values of < 12%, of 12%-13%, and of > 13% all clustered with *A. marmorata* in the phylogenetic tree with 100% bootstrap probability (Fig. 19), indicating that the proposed

species-specific differences in ADL/%TL may just be a phenotypic variation of *A. marmorata*, and the use of ADL/%TL to distinguish *A. luzonensis* and/or *A. celebesensis* might not be reliable.

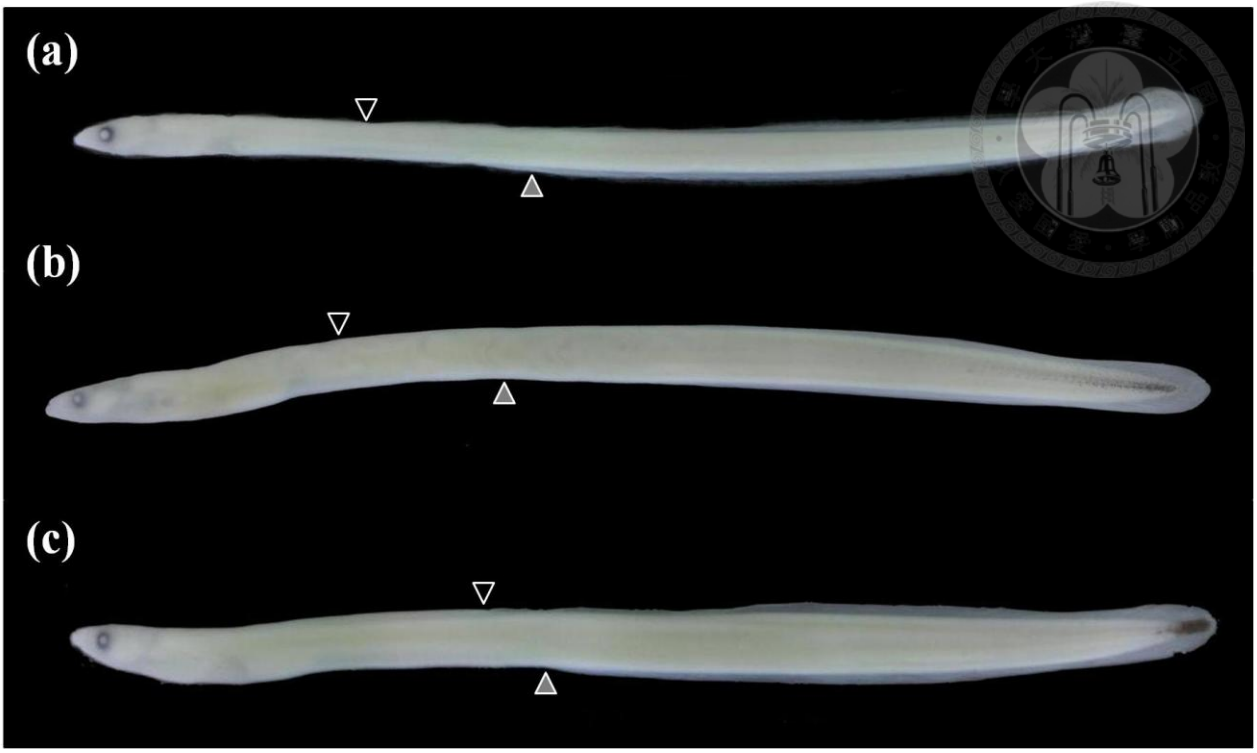


Fig. 17. Origins of the dorsal fin (black triangle) and anal fin (gray triangle) in longfin (a, *A. japonica* and b, *A. marmorata*, *A. celebesensis* and *A. luzonensis*) and shortfin (c, *A. bicolor pacifica*) glass eels.

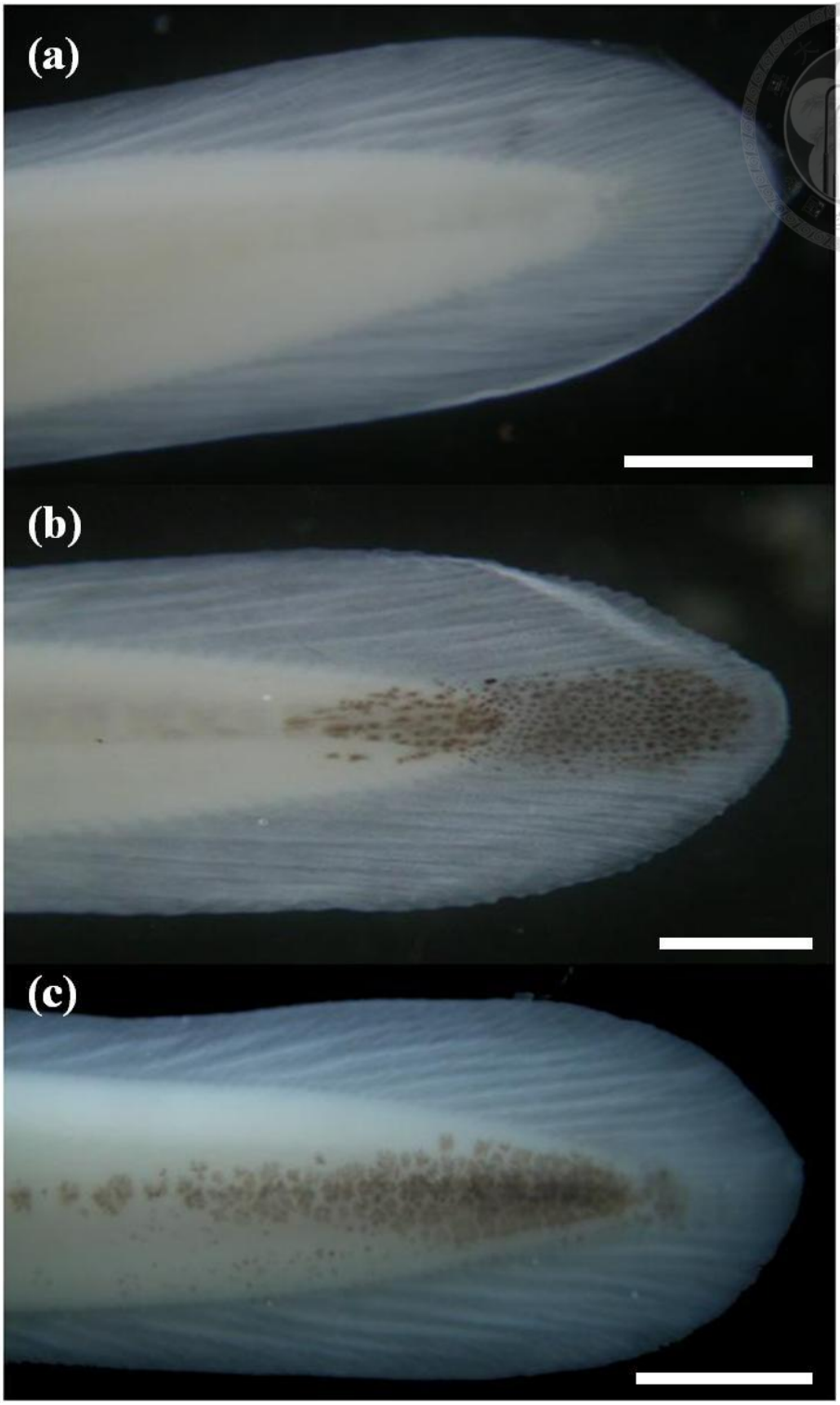


Fig 18. Caudal fin and tail bud pigmentation patterns of anguillid glass eels. (a) *Anguilla japonica*, (b) *A. bicolor pacifica* and (c) *A. marmorata*, *A. luzonensis* and/or *A. celebesensis*. Scale bar = 1.5 mm.

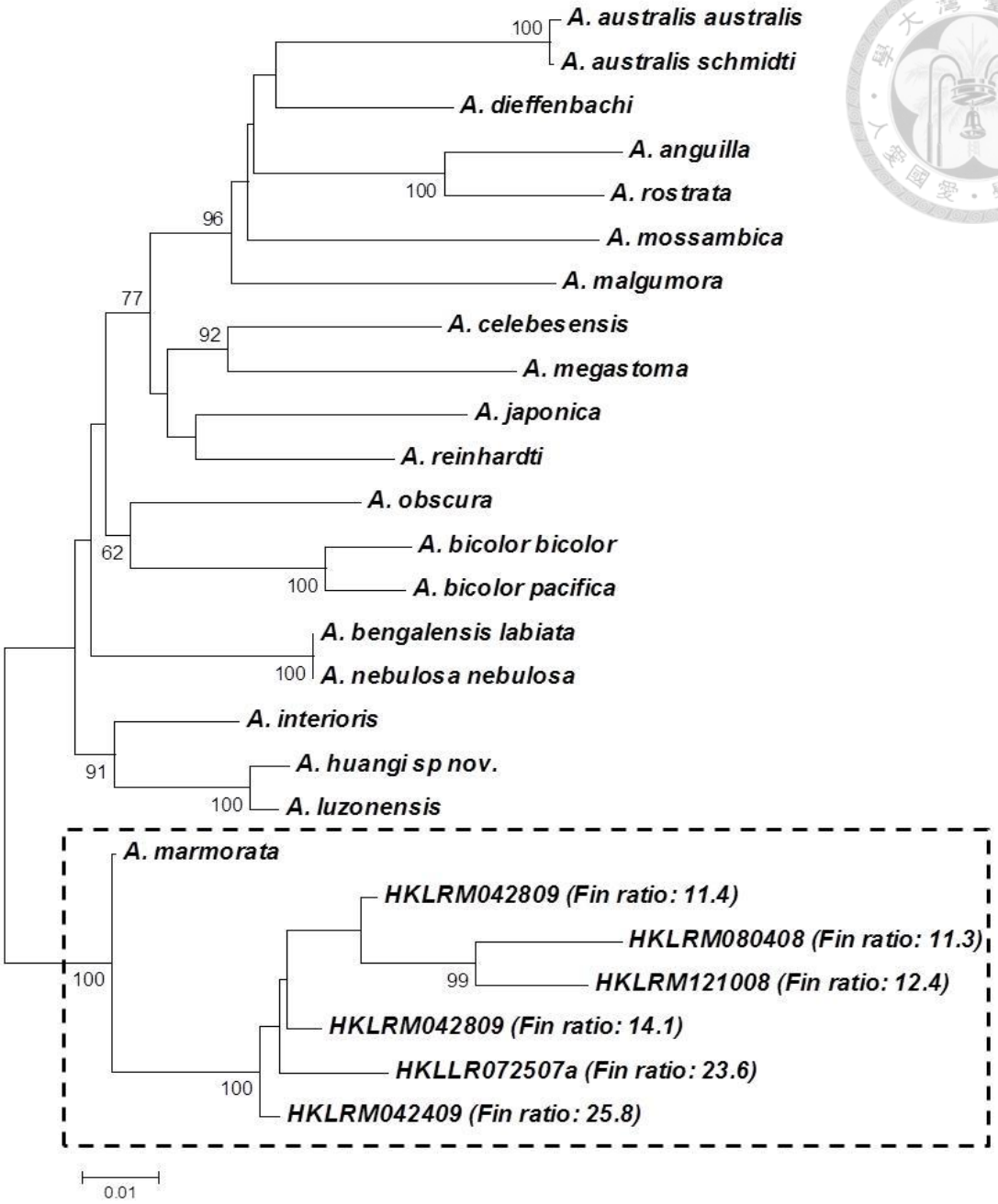


Fig. 19. Phylogenetic tree of the genus *Anguilla* inferred from cytochrome *b* sequences. Individuals with maximum (25.8% and 23.6%), medium (14.1% and 12.4%) and minimum (11.3% and 11.4%) values of the ratio of the ano-dorsal length to the percent total length (ADL/%TL) are also shown inside the dashed box. Bootstrap probabilities are indicated near the nodes.

3.2 Species composition of the glass eel recruited to Hsiukuluan River

The species composition of the 1004 glass eels collected in the estuary of Hsiukuluan River, eastern Taiwan was shown by month and year in Table 4 and by sampling station as shown in Fig. 20. Relative abundances of glass eels varied with sampling stations, months, and years (Table 4). Four anguillid eel species were identified based on caudal pigmentation patterns and morphometric measurements. *Anguilla japonica* is a temperate species, and relatively few (< 1%) were found in eastern Taiwan, although it is abundant on the west coast of Taiwan. *Anguilla marmorata* was most abundant among the 3 tropical eel species, making up 98.4% of the total catch, followed by a very few (1.3%) *A. bicolor pacifica*. But because the phylogenetic analysis did not support the occurrence of the new species and/or *A. celebesensis,* the relative abundance of *A. marmorata* should reach 98.4%.

It was found that different eel species recruit to the Hsiukuluan River at different times of the year: *A. marmorata* recruited mainly during spring and summer but was found year-round, while *A. bicolor pacifica* recruited during autumn. The temperate species, *A. japonica*, recruited mainly during winter.

Table 4. Species composition of *Anguilla* glass eels collected in the lower reach of Hsiukuluan River in Eastern Taiwan from 2005-2009. (A) and (B) indicate possible proportions of *A. marmorata* and *A. luzonensis* and/or *A. celebesensis* (uncertain). Values in parenthesis indicate the number of individuals used for the measurement of ADL/%TL to discriminate *A. marmorata* and *A. luzonensis* and/or *A. celebesensis* and the number of individuals used for molecular identification in A and B.

Sampling period		Total	Species composition					
			A. japonica	*A. bicolor pacifica*	*A. marmorata* (A) + uncertain (B)	A	B	Unidentified
2005	June	151	0	0	151	-	-	-
2007	July	26	0	0	26 (29)	24	1	1
	Sept.	8	0	0	8 (8)	6	1	1
2008	Apr.	1	0	0	1 (1)	1	0	0
	May	48	0	0	48 (48)	46	1	1
	June	54	0	0	54 (14)	12	0	2
	July	13	0	0	13 (13)	11	1	1
	Aug.	56	0	0	56	-	-	-
	Sept.	5	0	0	5 (5)	4	1	0
	Oct.	36	0	0	36 (22)	17	2	3
	Nov.	280	2	10	268 (67)	56	5	6
	Dec.	62	1	2	59 (54)	38	9	7
2009	Jan.	1	1	0	0	0	0	0
	Feb.	1	0	0	1	-	-	-
	Mar.	44	0	0	44 (24)	19	0	5
	Apr.	64	1	0	63 (60)	53	5	2
	May	17	0	0	17 (9)	9	0	0
	June	61	0	1	60 (48)	41	1	6
	July	69	0	0	69 (18)	17	0	1
	Aug.	4	0	0	4 (4)	2	0	2
	Sept.	3	0	0	3 (3)	2	0	1
Total		1004	5	13	986 (424)	358 (2)	27 (4)	39

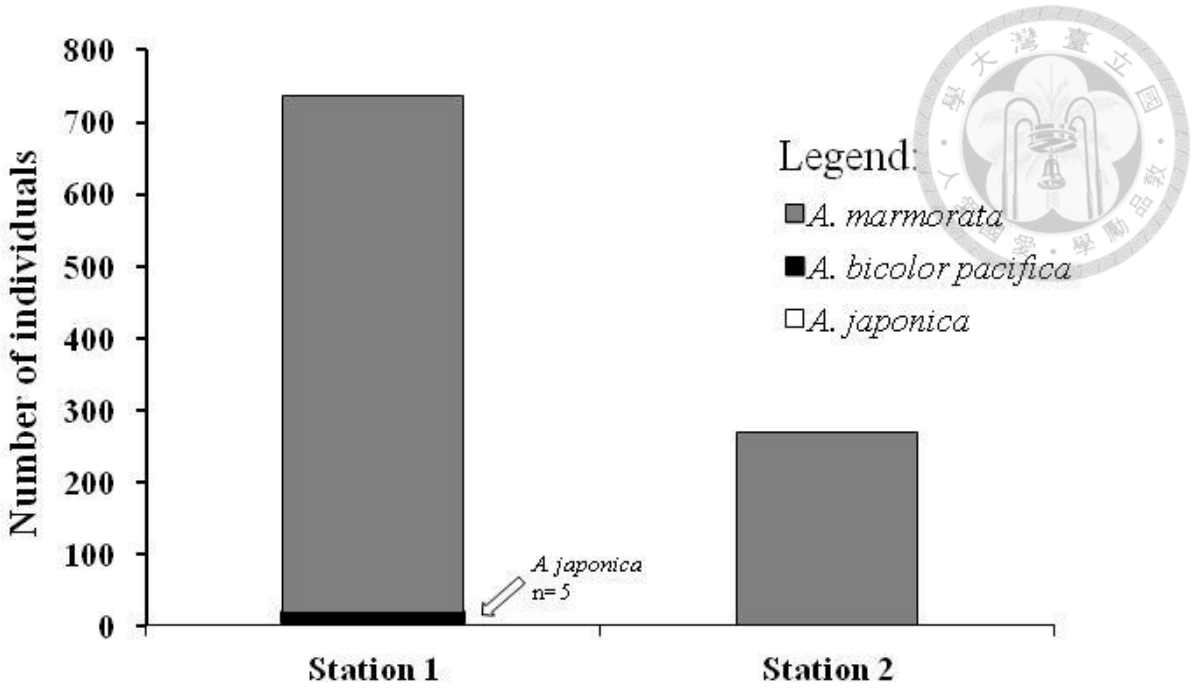


Fig. 20. Species composition of the recruiting glass eels collected in 2 stations at the lower reach of Hsiukuluan River, Eastern Taiwan from 2005-2009.

3.3 Stage composition of the glass eel recruited to Hsiukuluan River

The glass eels at arrival in the estuary of Hsiukuluan River were at the transition stage from glass eel to elver with various pigmentation stages (Table 5). At station 1 (river mouth), the majority of individuals caught were at stage V_A (62%) followed by stages V_B (34.1%), VI_{A1} (2.2%), and VI_{A2} (1.7%) (Fig. 21). These stages were characterized by the respective absence (stage V_A) or presence (stage V_B) of a cerebral nerve-cord spot and pigmentation on the tail and caudal fin (Bertin 1956). The pigmentation stage suggested that the glass eels had recently arrived at the river mouth. On the other hand, at station 2 (approximately 2 km upstream from the river mouth), the majority of individuals caught were at stage VI_{A1} (35.2%) followed by stages V_B (27.8%), VI_{A2} (13%), VI_{A4} (11.1%), VI_{A3} (7.4%), V_A (3.7%), and VI_B (1.9%) (Fig. 23). Stages V_B~VI_{A4} (elver stage) were characterized by a progressive pigmentation on the dorsolateral part of the elver.

Table 5. Pigmentation stages of the different *Anguilla* eel species collected from 2 stations in the lower reach of Hsiukuluan River.

Station	Species	n	Pigmentation stage						
			V_A	V_B	VI_{A1}	VI_{A2}	VI_{A3}	VI_{A4}	VI_B
1	*A. japonica*	5	5	0	0	0	0	0	0
	A. bicolor pacifica	12	11	1	0	0	0	0	0
	A. marmorata	144	85	53	4	2	0	0	0
	Uncertain	18	10	7	0	1	0	0	0
	Total	179	111	61	4	3	0	0	0
	% composition		62.0	34.1	2.2	1.7	0	0	0
2	*A. japonica*	0	0	0	0	0	0	0	0
	A. bicolor pacifica	1	0	0	0	0	0	0	0
	A. marmorata	44	2	11	17	7	1	5	1
	Uncertain	9	0	3	2	0	3	1	0
	Total	54	2	14	19	7	4	6	1
	% composition		3.7	27.7	35.2	13.0	7.4	11.1	1.9

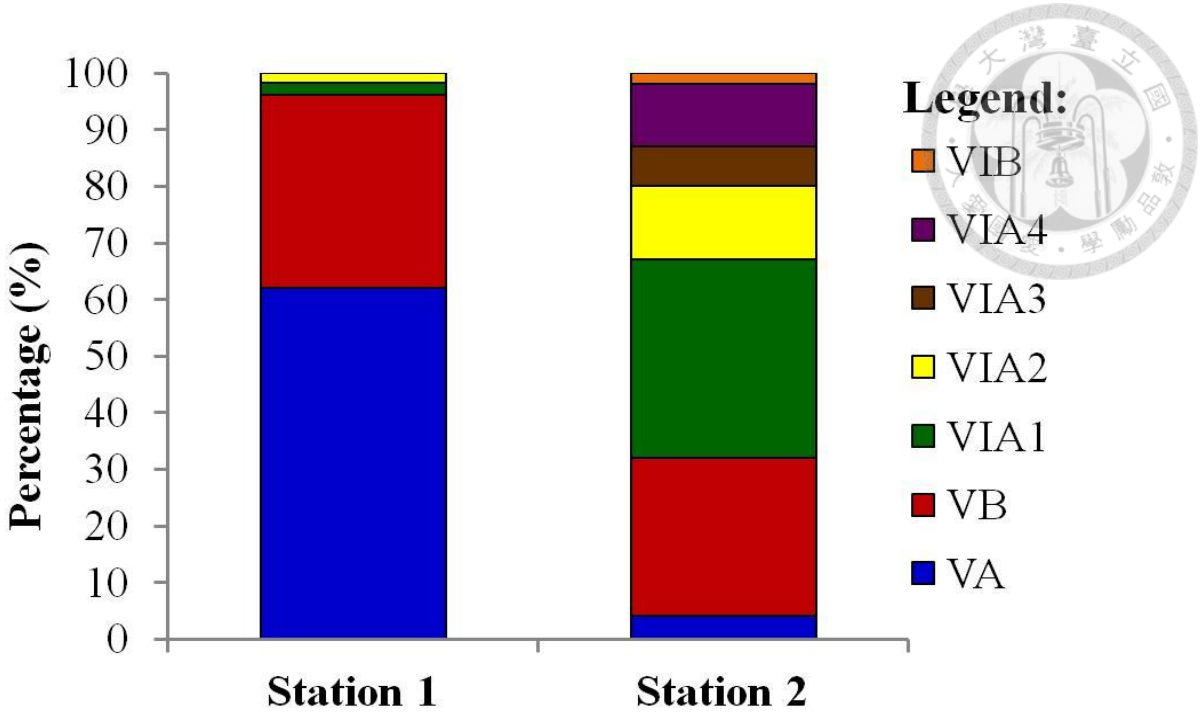


Fig 21. Developmental stage composition of recruiting glass eels according to sampling station.

3.4 Comparison of morphometric characters of glass eels among species

Based on groupings according to caudal cutaneous pigmentation patterns, morphometric measurements of different species were determined. Because the molecular analysis did not support the occurrence of *A. luzonensis* and/or *A. celebesensis* in the sample examined, individuals with ADL/%TL values of < 13 were now classified as *A. marmorata* and were not included in the morphometric comparisons. Ranges and mean TLs differed among eel species (Table 6). TLs of long-finned eels ranged 46.47-58.04 (mean ± standard deviation: 49.43 ± 2.32) mm in *A. marmorata*, 45.45-57.79 (53.01 ± 4.54) mm in *A. japonica*, and 40.40-47.00 (44.50 ± 3.04) mm in *A. celebesensis* (Tzeng 1982). On the other hand, the TL of *A. bicolor pacifica* ranged 45.86-49.22 (47.29 ± 1.06) mm. TLs of all 4 species examined significantly differed (ANOVA, $p < 0.05$). Length-frequency distributions of all species are presented in Fig. 22. *Anguilla japonica* had the largest mean TL, followed by *A. marmorata*, *A. bicolor pacifica*, and *A. celebesensis*.

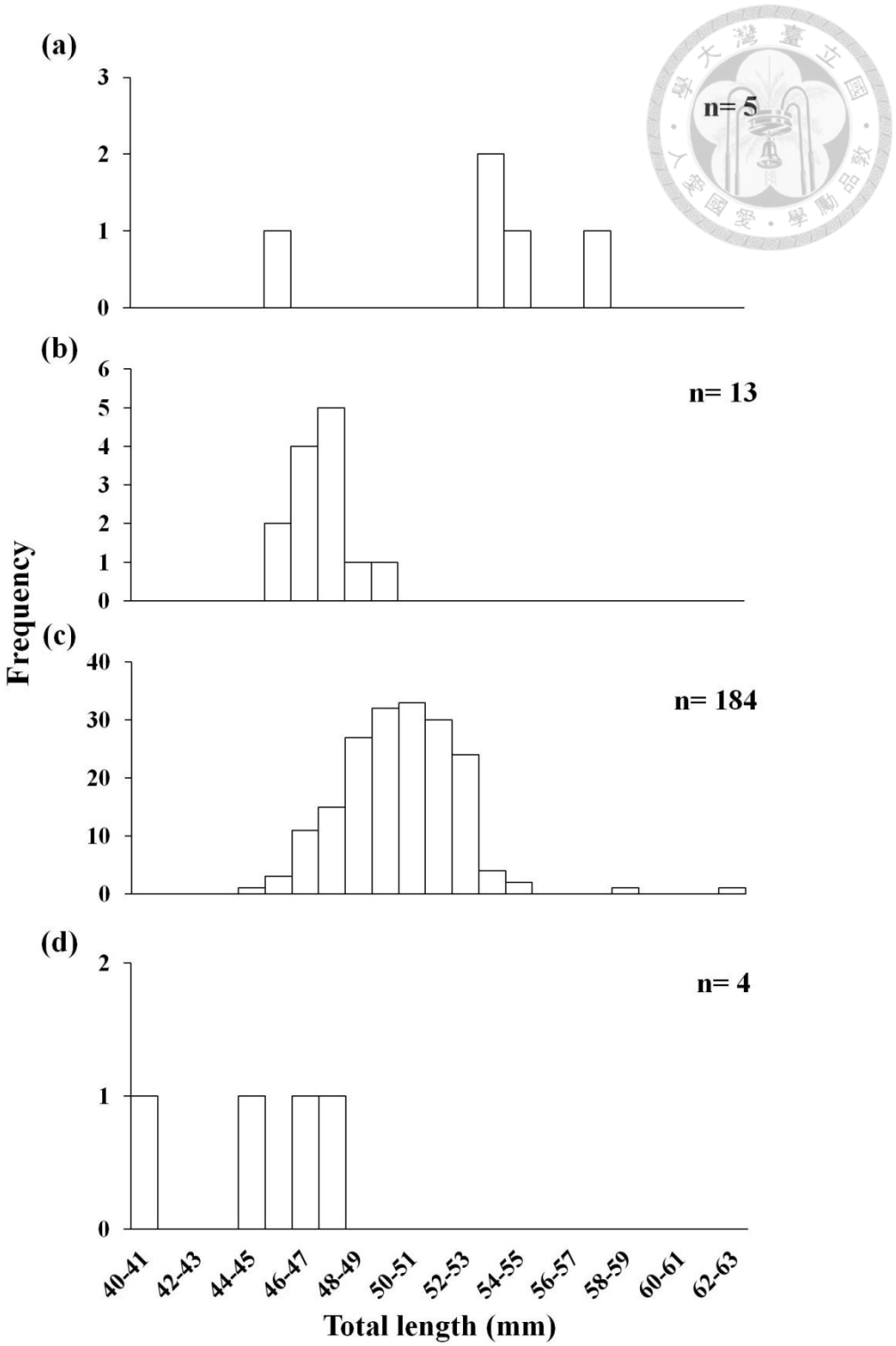


Fig. 22. Length frequency distribution of glass eels of *Anguilla japonica* (a), *A. bicolor pacifica* (b), and *A. marmorata* (c) caught in the lower reach of Hsiukuluan River of Eastern Taiwan and *A. celebesensis* (d) (Tzeng 1982). n= sample size.

The PDL of the short-finned eel *A. bicolor pacifica* ranged 16.52-18.55 (17.82 ± 0.63) mm, the largest among all eel species examined (Table 6). In long-finned eel species, the PDL ranged 12.04-15.05 (13.21 ± 1.59) mm in *A. japonica*, 9.00-14.40 (11.43 ± 0.94) mm in *A. marmorata*, and 12.50-13.70 (13.03 ± 0.50) mm in *A. celebesensis*. Significant differentiation in PDL was observed among species (Kruskal-Wallis: $H = 36.51$, d.f. = 3, $p < 0.001$; Table 4). No significant differentiation was observed between *A. bicolor pacifica* and *A. japonica* or between *A. celebesensis* and *A. marmorata* ($p > 0.05$; Table 6). *Anguilla marmorata* was observed to have the smallest PDL, while *A. bicolor pacifica* had the largest. PAL, on the other hand, did not significantly differ between short- and long-finned eel species ($p > 0.05$; Table 6).

The PAL of *A. bicolor pacifica* ranged 16.77-18.74 (18.04 ± 0.60) mm, while those of *A. japonica*, *A. marmorata,* and *A. celebesensis* ranged 15.85-19.46 (18.54 ± 1.56), 17.02-23.92 (19.13 ± 1.32), and 15.80-19.40 (17.65 ± 1.58) mm, respectively. No significant differentiation in PAL was observed among species (ANOVA, $p > 0.05$; Table 6).

According to the ADL, anguillid eels collected were classified into a short-finned eel, i.e., *A. bicolor pacifica*, with a mean (± SD) ADL of 0.22 ± 0.11 (0.08-0.37) mm, which was significantly smaller than those of long-finned eels, *A. japonica* (mean, 5.33 ± 1.43; range, 3.81-7.02 mm), *A. marmorata* (7.70 ± 0.95; 6.20-9.91 mm), and *A. celebesensis* (4.63 ± 1.16; 3.30-5.70 mm; Tzeng 1982) (Table 6). *Anguilla marmorata* had the largest ADL followed by *A. japonica*, *A. celebesensis*, and *A. bicolor pacifica*. Significant differentiation in ADL was observed among species (Kruskal-Wallis, $H = 40.18$, d.f. = 3, $p < 0.001$; Table 6). No significant difference was observed between *A. celebesensis* and *A. marmorata* or between *A. bicolor pacifica* and *A. japonica* ($p > 0.05$; Table 6).

The ADL/%TL value of *A. marmorata* ranged 13.27%-20.35% (15.57% ± 1.77%) while values of the other eel species ranged 0.17%-0.79% (0.43% ± 0.22%) in *A. bicolor pacifica*, 8.19%-12.97% (10.03% ± 2.43%) in *A. japonica*, and 12.13%-18.17% (10.30% ± 1.97%) in *A. celebesensis*. Significant differentiation in ADL/%TL values was observed (Kruskal-Wallis, H = 40.16, d.f. = 3, $p < 0.001$; Table 6). No significant difference was found between *A. celebesensis* and *A. marmorata* or between *A. japonica* and *A. bicolor pacifica* ($p > 0.05$; Table 6).

Figure 23-24 indicates that the short-finned eel *A. bicolor pacifica* can easily be distinguished from *A. marmorata*, *A. japonica*, and *A. celebesensis* using body proportions, especially the PDL and ADL in relation to TL. The PDL/TL (%) (Fig. 23) was 36.02%-37.69% (mean, 37.68%) in the short-finned eel *A. bicolor pacifica*, while in the long-finned eel species it ranged 19.37%-24.81% (23.12%) in *A. marmorata*, 26.49%-26.04% (24.92%) in *A. japonica*, and 27.68%-30.94% (29.28%) in *A. celebesensis*. On the other hand, ADL/TL (%) more-significantly differed between short- and long-finned eel species ($p < 0.05$, Table 6). The ADL/TL (%) (Fig. 24) was 0.17%-0.75% (0.46%) in the short-finned eel, *A. bicolor pacifica*, while in the long-finned eel species it ranged 13.34%~17.07% (15.58%) in *A. marmorata*, 8.38%-12.15% (10.05%) in *A. japonica*, and 8.17%-12.13% (10.40%) in *A. celebesensis*.

Table 6. Comparison of morphometric characters among the 4 *Anguilla* species. All values are in millimeters except for ADL/%TL. Values inside parentheses beside the species names indicate the sample sizes used for morphometric comparisons. Different superscript letters indicate a significant difference at $p < 0.05$. *Measurements from Tzeng (1982).

	Mean ±SD (range) mm			
	A. japonica (5)	*A. bicolor pacifica* (13)	*A. marmorata* (30)	*A. celebesensis** (4)
Total length	53.01±4.54 (45.45-57.79)^{d}	47.29±1.06 (45.86-49.22)^{b}	49.43±2.32 (46.47-58.04)^{c}	44.50±3.04 (40.40-47.00)^{a}
Pre-dorsal fin length	13.21±1.59 (12.04-15.05)^{b}	17.82±0.63 (16.52-18.55)^{b}	11.43±0.94 (9.00-14.40)^{a}	13.03±0.50 (12.50-13.70)^{a}
Pre-anal fin length	18.54±1.56 (15.85-19.46)^{a}	18.04±0.60 (16.77-18.74)^{a}	19.13±1.32 (17.02-23.92)^{a}	17.65±1.58 (15.80-19.40)^{a}
Ano-dorsal fin length	5.33±1.43 (3.81-7.02)^{a}	0.22±0.11 (0.08-0.37)^{a}	7.70±0.95 (6.20-9.91)^{b}	4.63±1.16 (3.30-5.70)^{b}
ADL/%TL	10.03±2.43 (8.19-12.97)^{a}	0.43±0.22 (0.17-0.79)^{a}	15.57±1.77 (13.27-20.35)^{b}	10.30±1.97 (8.17-12.13)^{b}

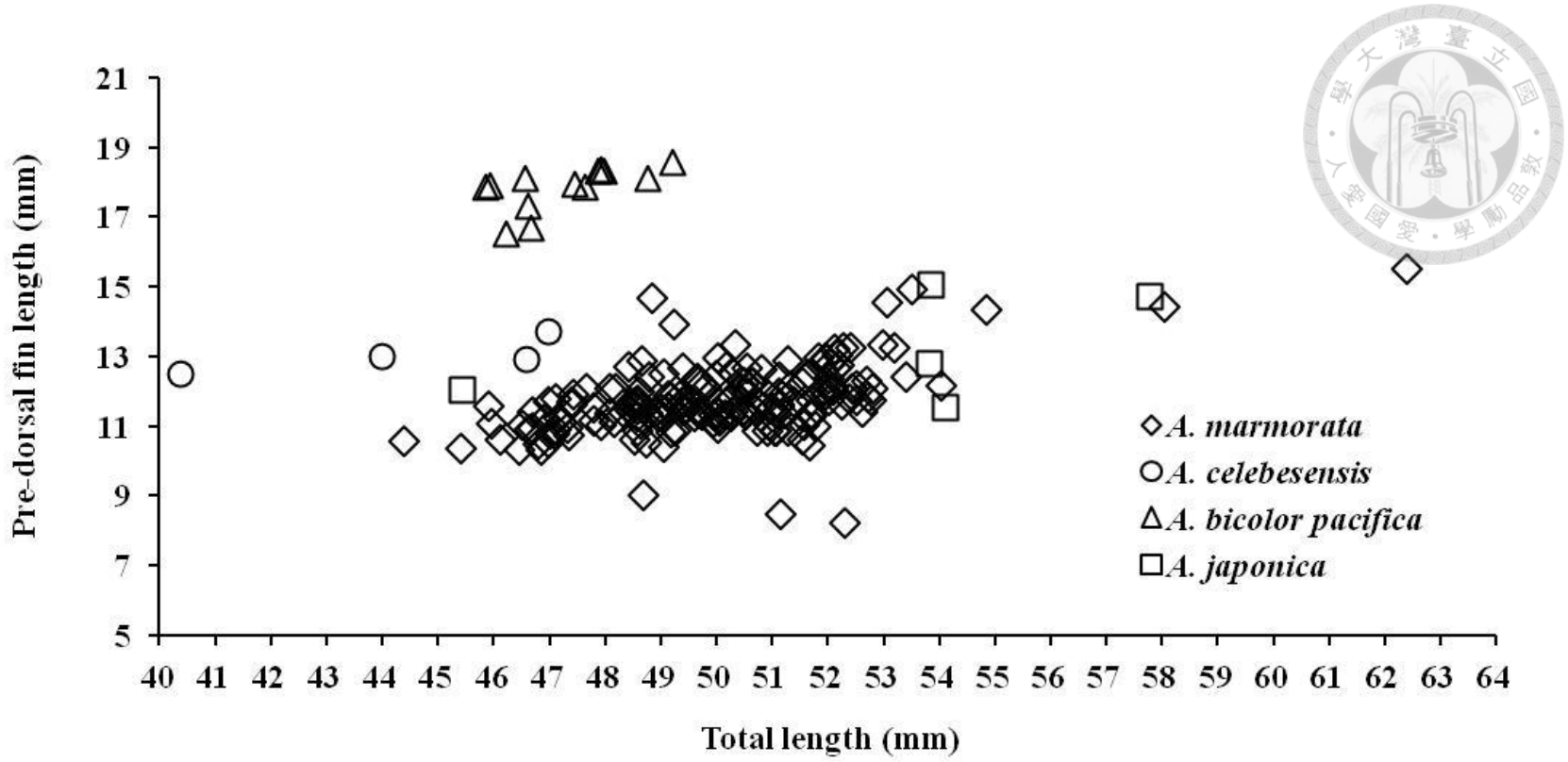


Fig. 23. Relationship between pre-dorsal fin length and total length in the glass eels of *Anguilla japonica*, *A. bicolor pacifica*, *A. marmorata* and *A. celebesensis* (from Tzeng 1982).

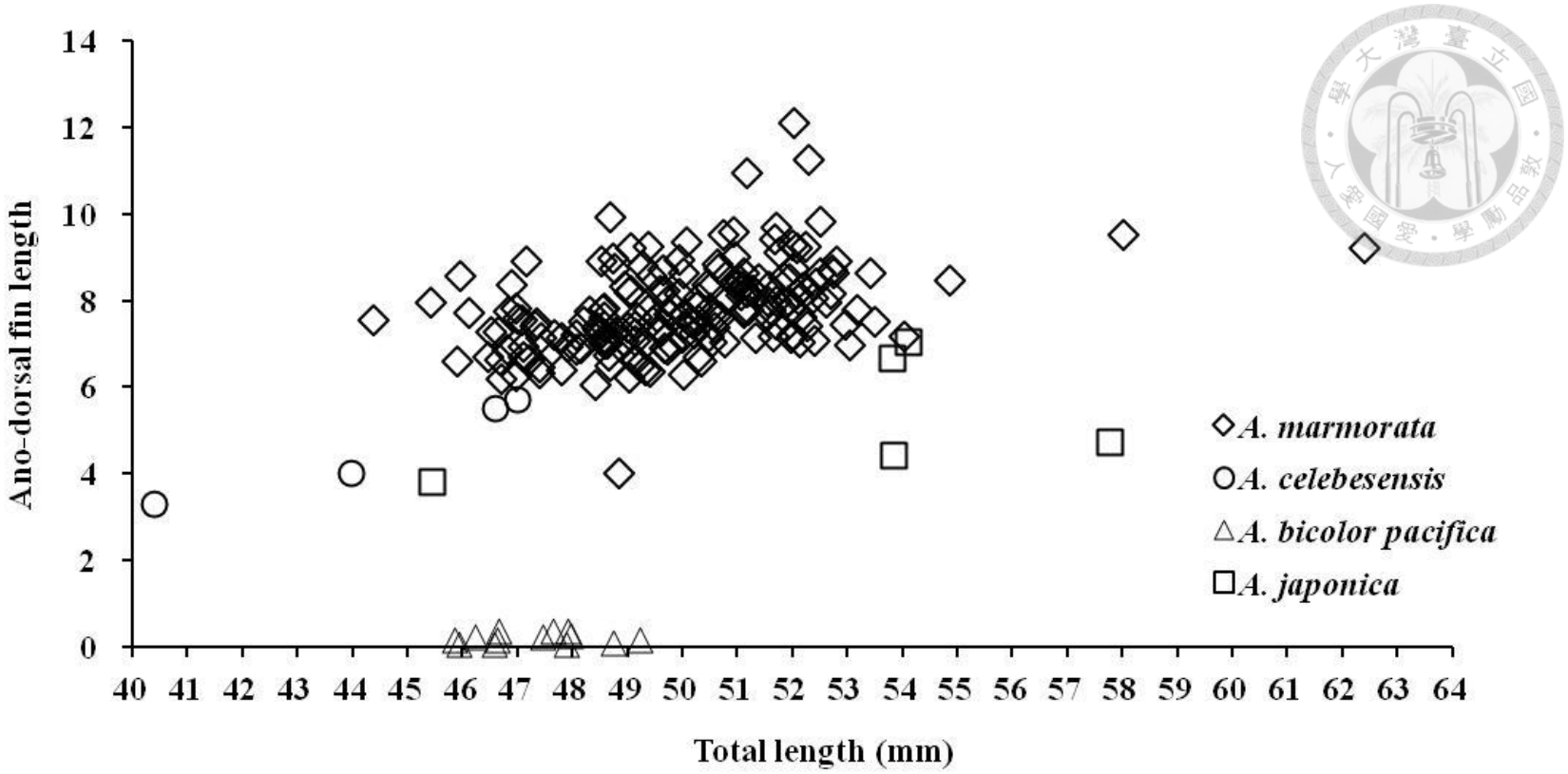


Fig. 24. Relationship between ano-dorsal fin length and total length in the glass eels of *Anguilla japonica*, *A. bicolor pacifica*, *A. marmorata* and *A. celebesensis* (from Tzeng 1982).

3.5 Daily growth increments and growth checks in the otoliths of glass eel of *Anguilla marmorata*

Otolith microstructures between the temperate *A. japonica* and tropical *A. marmorata* were fundamentally the same (Fig. 25). DGIs in otoliths of glass eels are composed of 2 layers called the incremental (L) and discontinuous (D) zones, which respectively appeared to be light and dark as revealed by the SEM photo in Fig. 25a. The L-zone is rich in calcium carbonate ($CaCO_3$), while the D-zone is very rich in protein but poor in calcium. When etched with hydrochloric acid (HCl) or EDTA and viewed under an SEM, the L-zone appeared elevated while the D-zone appeared as a ridge. A single DGI is usually composed of an L-zone and a D-zone and is generally deposited on a daily basis. Near the otolith edge, a distinct growth check called the elver check was found (Fig. 25b). The elver check was deposited in the elver stage during its migration from seawater to fresh water. It also marks the transition from the glass-eel to the elver stage. On the other hand, DGIs at the beginning of the leptocephalus stage were wide and clear but became very diffuse and obscure and almost uncountable near the metamorphosis area (Fig. 25c). This indicated that the leptocephalus grew fast during the early developmental stage, then gradually slowed down and reached an asymptotic length before metamorphosis. Thus an MC was deposited at the transition from the leptocephalus to glass-eel stage. After the MC to the otolith edge, the DGIs became wider, indicating that growth speeded up after metamorphosis. The P in the otolith of the elver was an amorphous structure which appeared as a deep hole after etching with HCl or EDTA (Fig. 25d). Distinct concentric growth increments and check rings were observed around the P that marked hatching (HC) and 1st feeding (FFC). The HC appeared as a deep circular grove surrounding the P. Between the HC and FFC, no distinct DGI was discernible and it is called embryonic-life band (Fig. 25d). From

the P to the otolith edge, the change in DGI widths revealed the growth history of the eel as it migrated from the oceanic spawning ground until it was recruited to the estuary (Fig. 25e). Also, the DGIs recorded different life-history and developmental-stage transitions. In all of the otoliths examined, P are very distinct and well-defined (Fig. 26a) and can vary from a round to a sub-elliptical shape but it can sometimes be vague and undefined (Fig. 26b). Primordium is always single but on rare occasions, it can be twin (Fig. 26c). These twin primordia coalesced to form the core of the otolith.

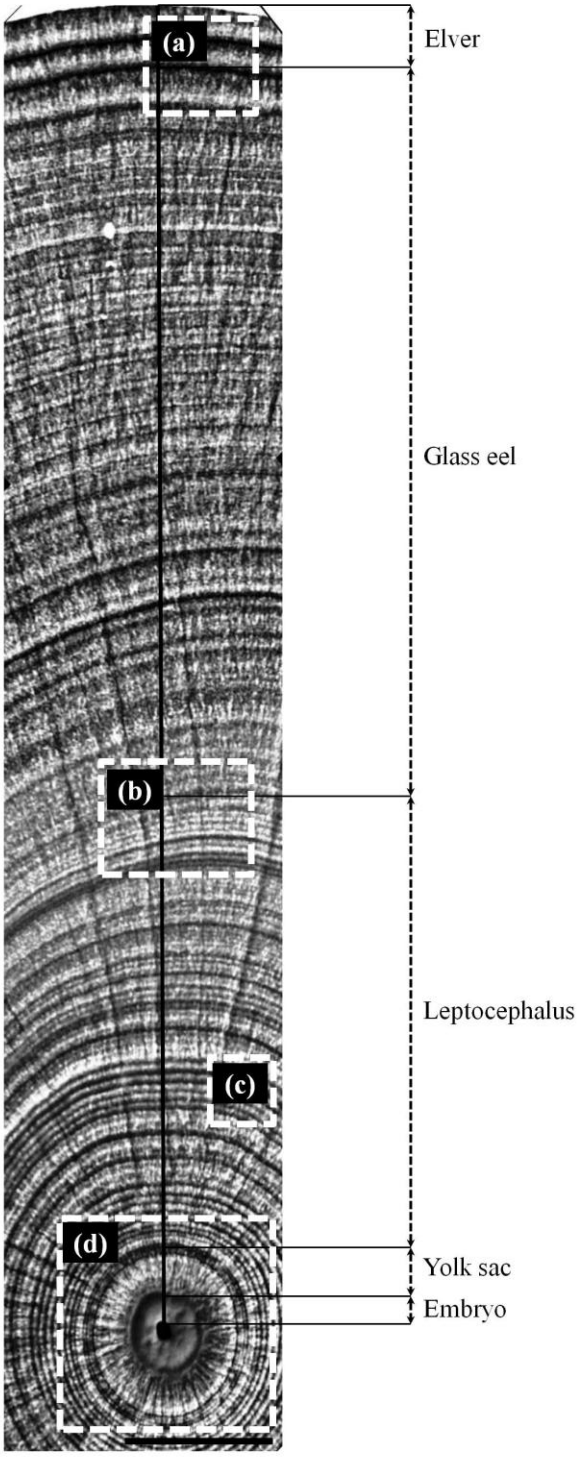


Fig. 25. SEM photographs showing daily growth increments (DGIs) and growth checks in an otolith of an *Anguilla marmorata* elver. Magnified portions of a to d is shown in the succeeding pages. Scale bar = 20 μm.

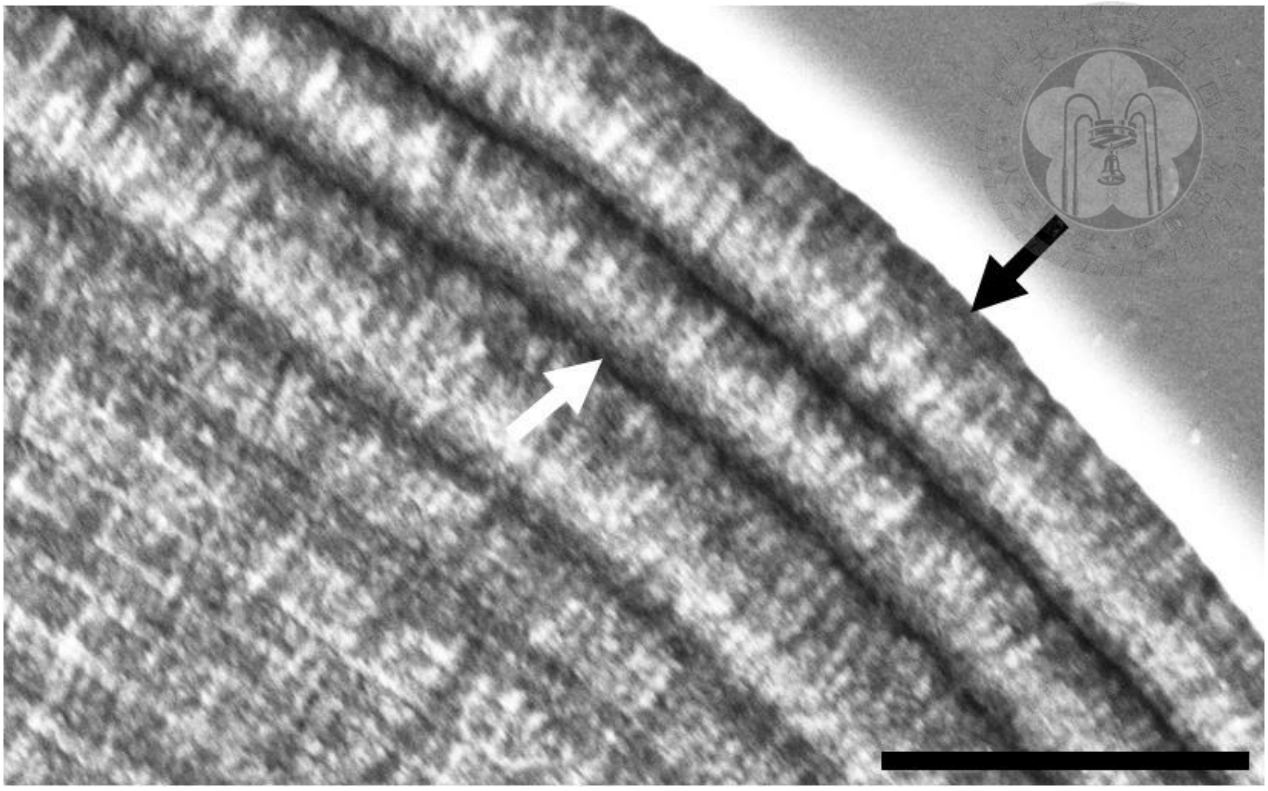


Fig. 25a. Peripheral region of the *Anguilla marmorata* glass eel otolith showing the estuarine check (white arrow) and the otolith edge (black arrow). Scale bar = 20 µm.

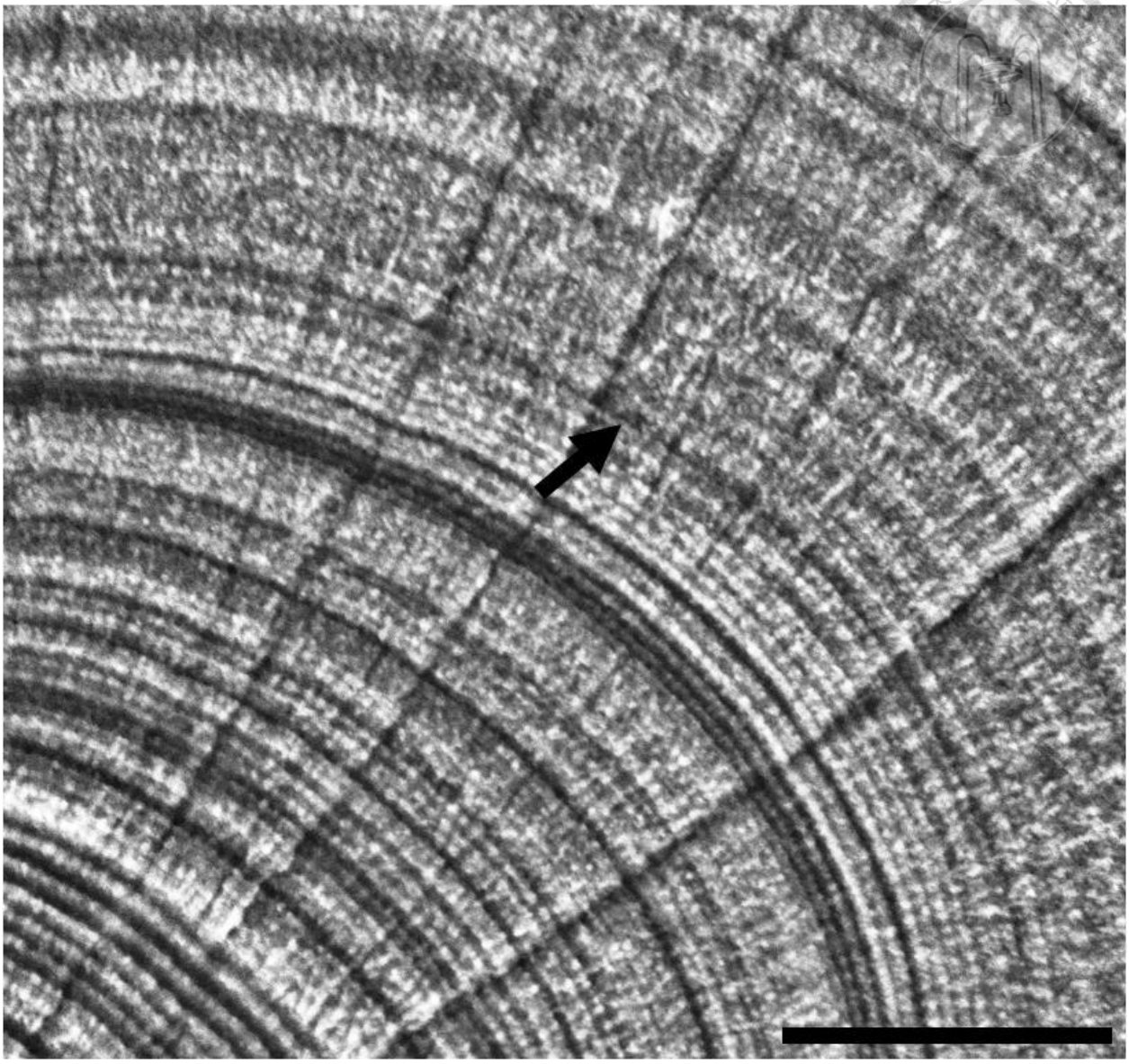


Fig. 25b. SEM photograph showing the metamorphosis check (black arrow) in the *Anguilla marmorata* glass eel otolith. Scale bar = 20 μm.

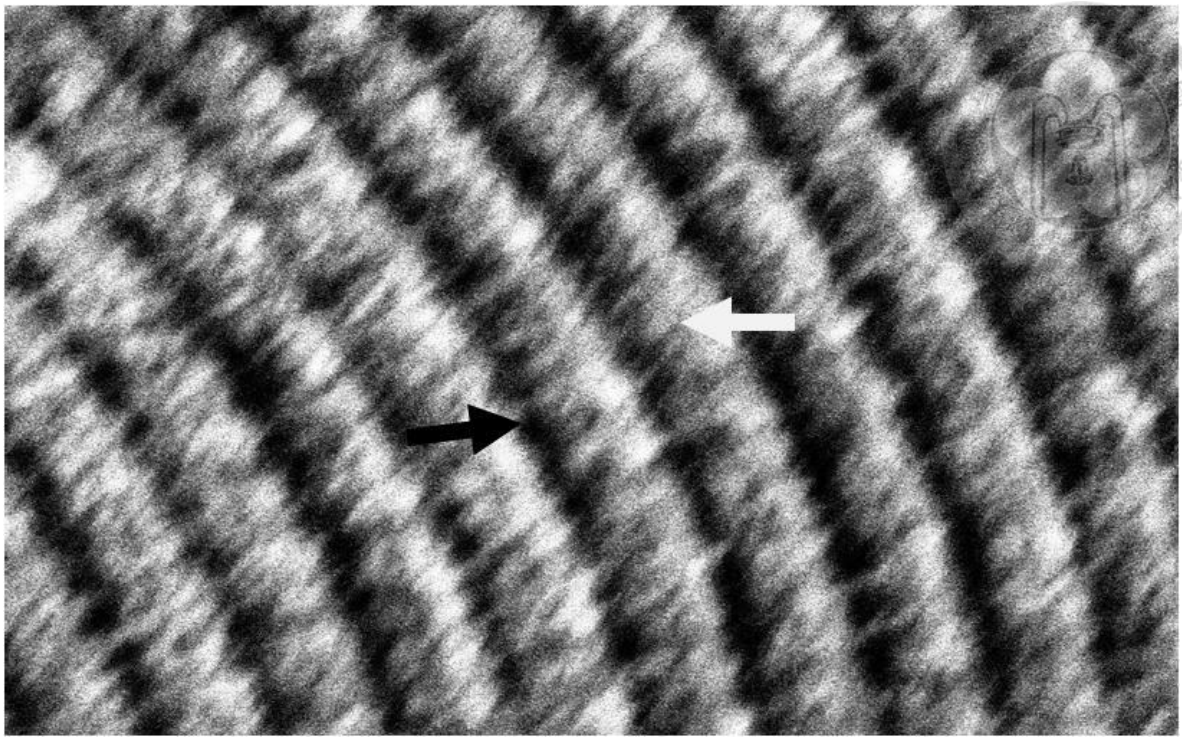


Fig. 25c. SEM photograph showing the discontinuous (dark band, black arrow) and increment (light band, white arrow) zones in the *Anguilla marmorata* glass eel otolith.

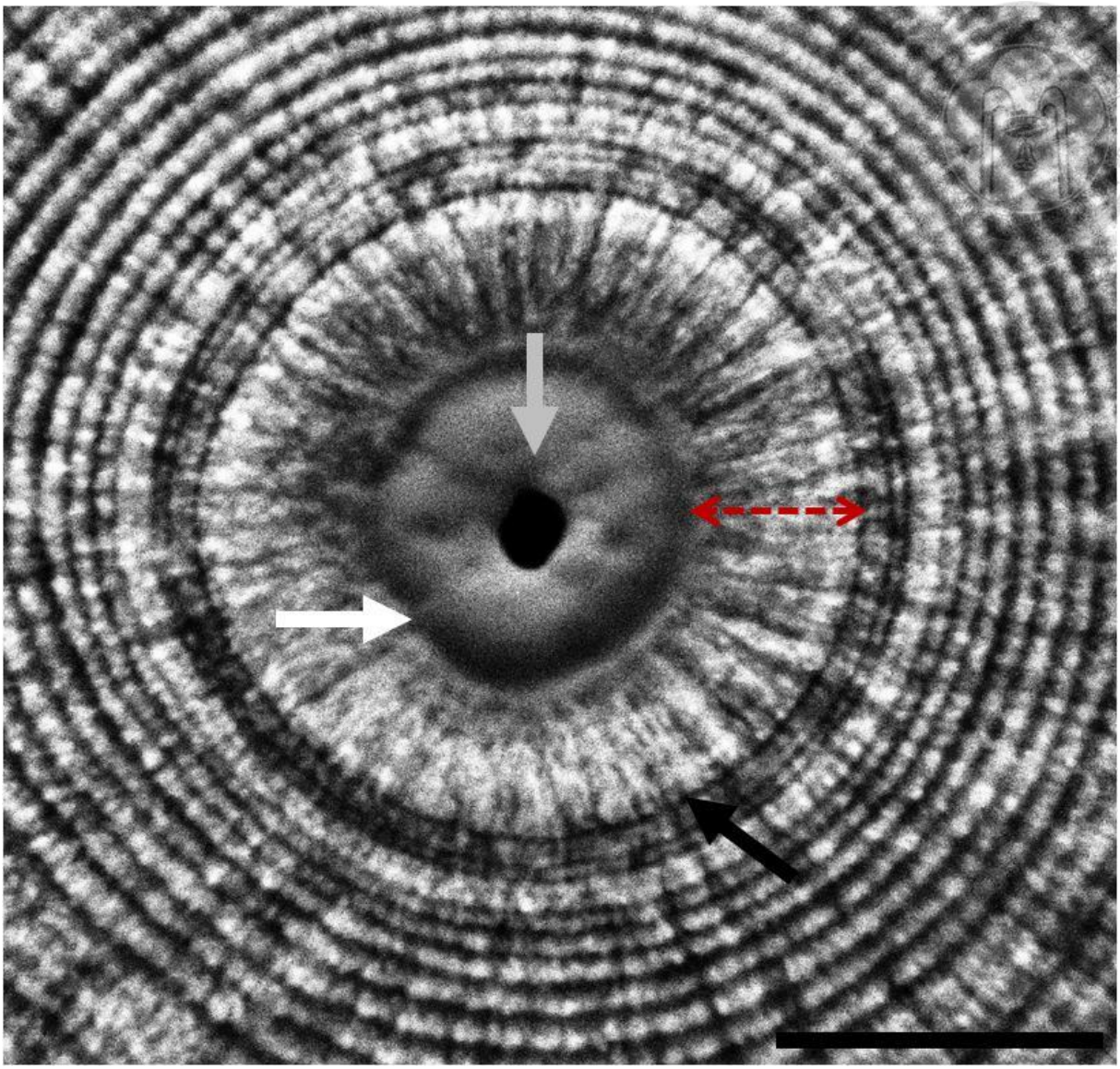


Fig. 25d. Core region of the of the *Anguilla marmorata* glass eel otolith showing the primordium (gray arrow), yolk-sac stage(red dashed line), hatching check (white arrow) and the 1st feeding check (black arrow). Scale bar = 20 µm.

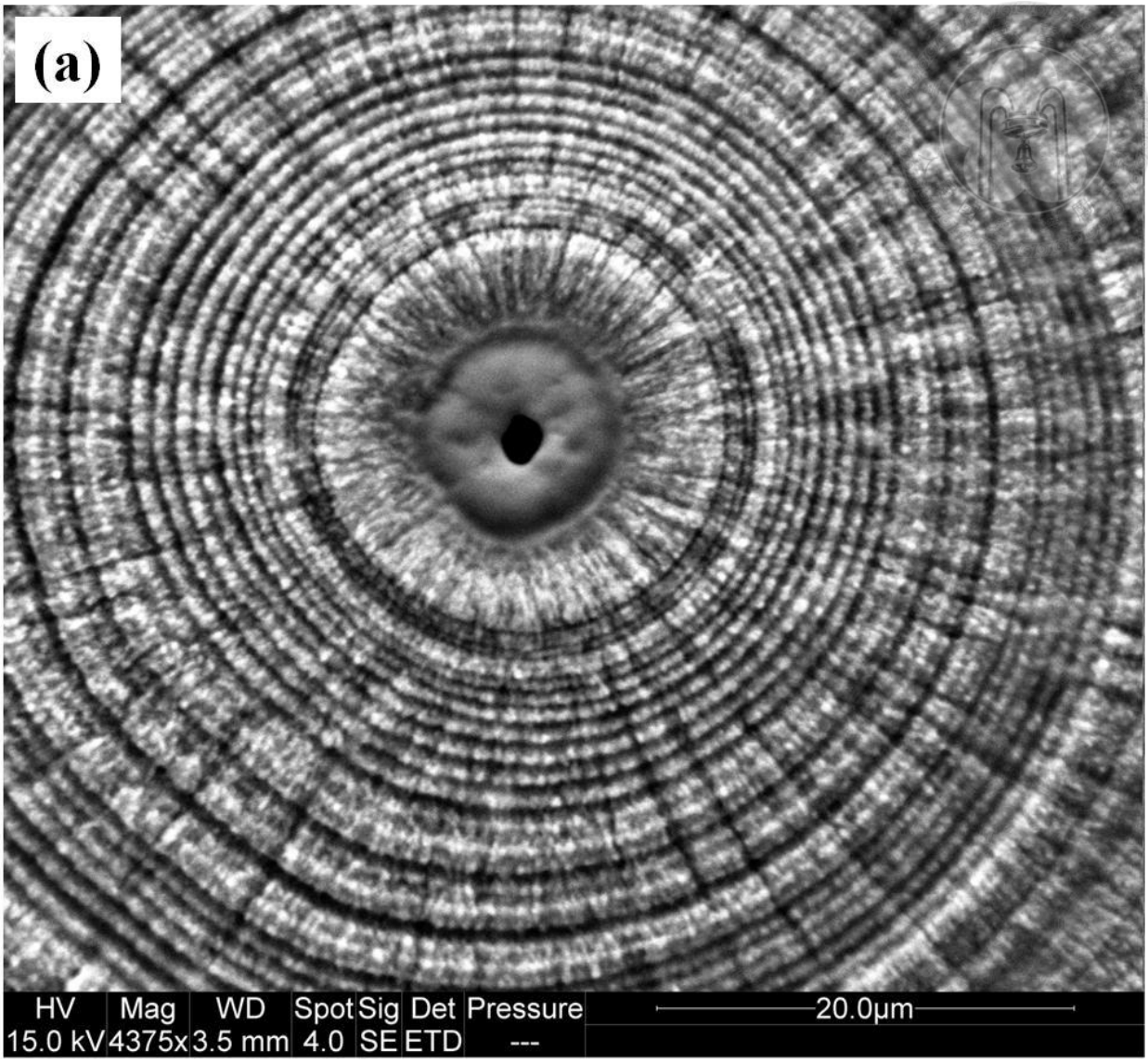


Fig. 26a. Core region of the *Anguilla marmorata* glass eel otolith showing a distinct and well-defined primordium.

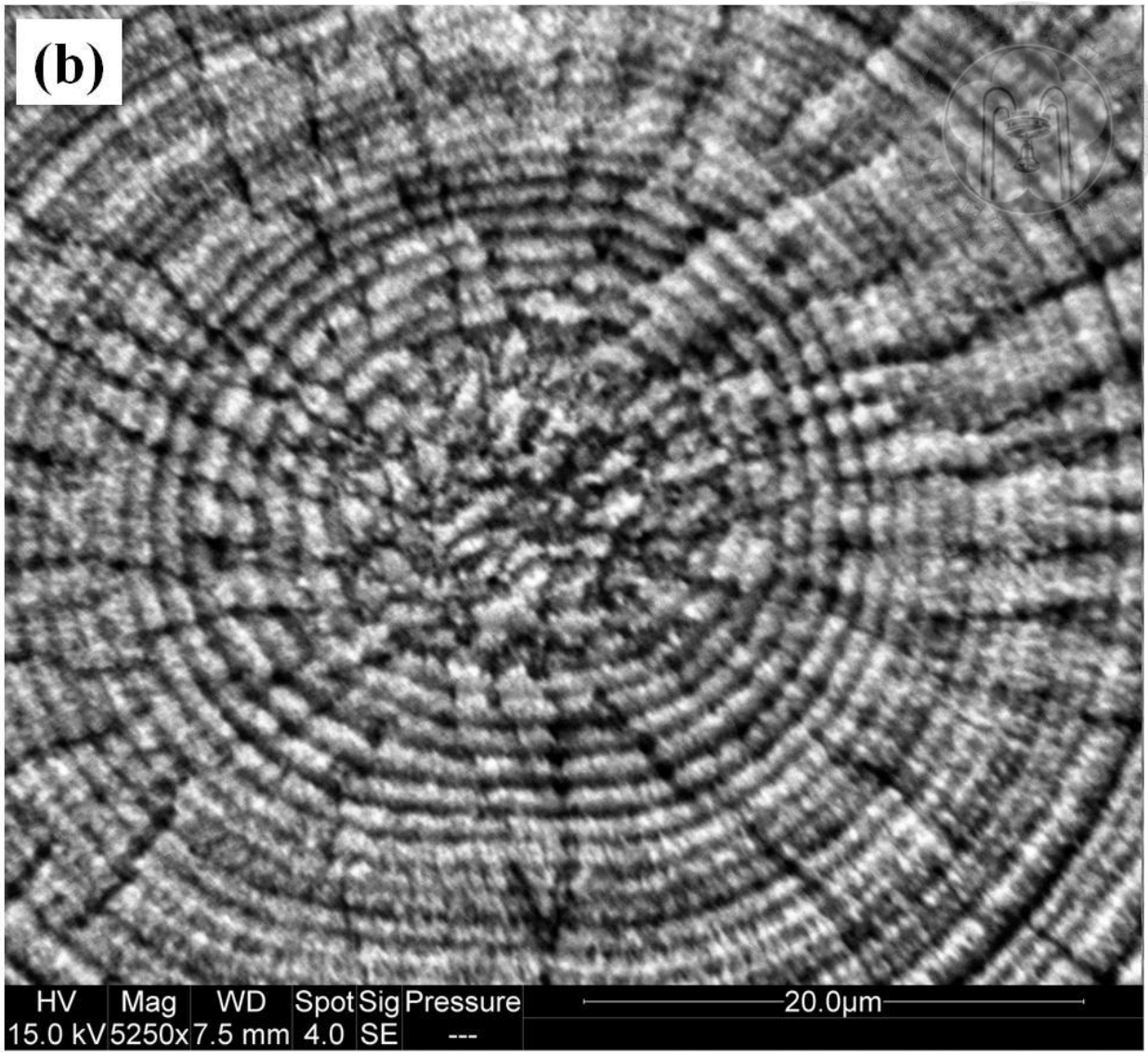


Fig. 26b. Core region of the *Anguilla marmorata* glass eel otolith showing an undefined primordium.

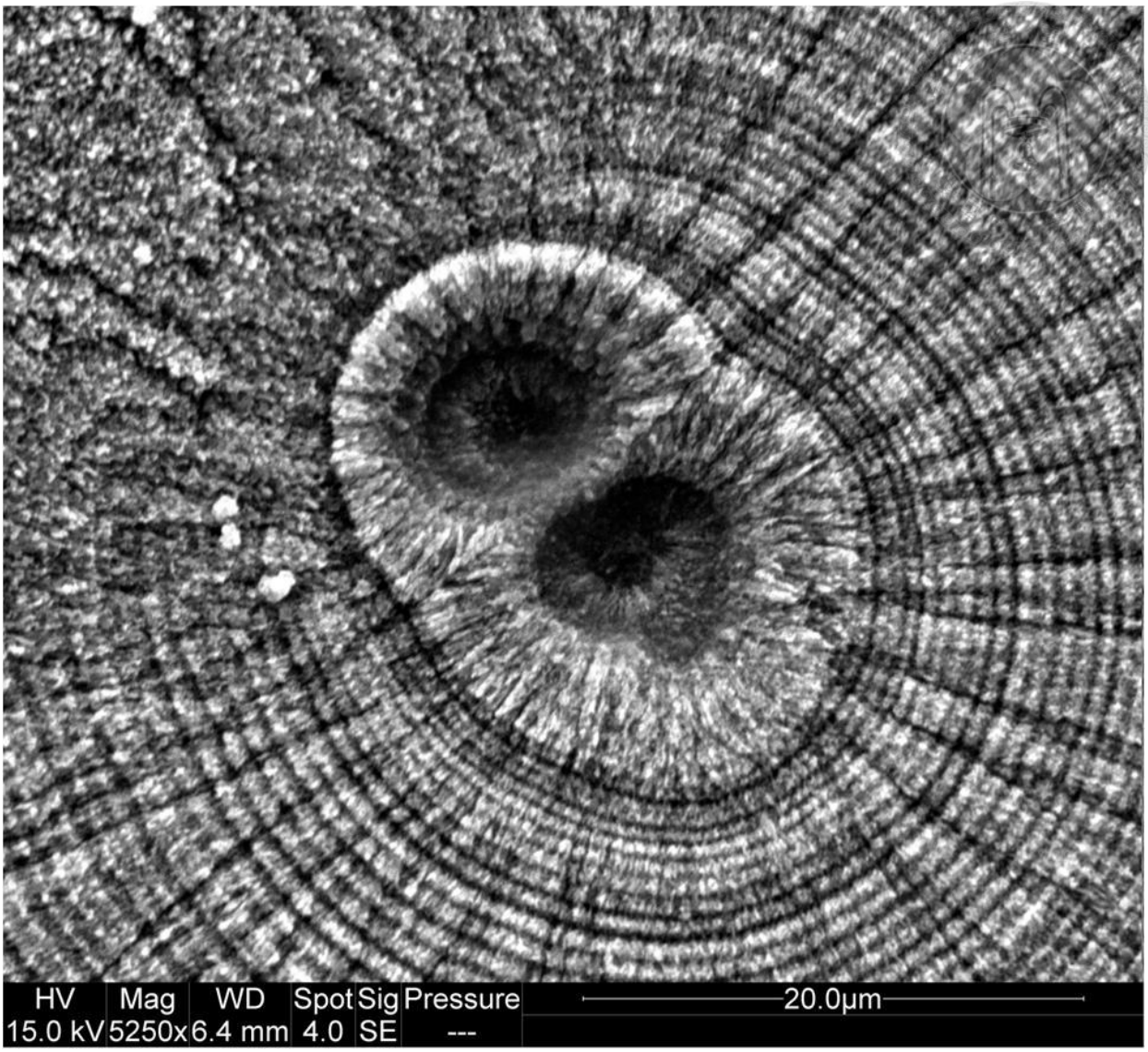


Fig. 26c. Core region of the *Anguilla marmorata* glass eel otolith showing twin primordia.

3.6 Comparison of the stage composition between *Anguilla marmorata* and *A. japonica* glass eels

The majority of *A. marmorata* collected and examined (*n* = 168) from various estuaries and rivers in East Asia were at stage V_A (55.4%) followed by stage V_B (44.6%) (Table 7; Fig. 27a). No *A. marmorata* in a more-advanced developmental stage (i.e., stages VI or VII) were observed. On the other hand, the majority of *A. japonica* examined (*n* = 240) were at stage V_A (51.7%) followed by stages V_B (32.1%), VI_{A1} (12.5%), VI_{A2} (3.3%), and VI_{A3} (0.4%). Also, the occurrence of larger *A. japonica* individuals in the Yalu River (Table 7) corresponded with their more-advanced pigmentation states (VI_{A2} and VI_{A3}) (Table 8; Fig. 27b).

Table 7. Total lengths, daily age of glass eels at the estuary (T_t), daily age at metamorphosis from leptocephalus to glass eel (T_m), and the time between the metamorphosis check and estuarine arrival ($T_{t\text{-}m}$) of *Anguilla japonica* and *A. marmorata*. Values inside parentheses indicate the number of individuals used for aging.

Species	Sampling site	n	Total length (mm)	Age (days)		
				T_m	T_t	$T_{t\text{-}m}$
*A. japonica**	Tung-kang River, TW	30 (16)	57.0±2.0	117.7±14.3	156.9±21.1	39.2±6.8
		30 (14)	56.1±2.4	121.4±12.0	164.3±18.2	42.9±6.2
	Shuang-shi River, TW	30 (12)	56.8±2.3	125.9±14.7	157.6±22.3	31.7±7.6
		30 (13)	55.9±2.2	115.8±8.1	154.7±13.9	38.9±5.8
	Ming-chiang River, CN	30 (20)	55.1±1.9	128.4±6.9	162.9±10.5	34.5±3.6
	Chyan-tarng River, CN	30 (23)	55.6±1.9	137.9±11.3	176.5±17.0	38.6±5.7
	Ya-lu River, CN	30 (23)	58.3±1.8	135.5±11.3	178.3±18.7	42.8±7.4
	Ichinomiya River, JP	30 (10)	57.4±2.3	137.0±12.9	182.0±22.1	45.0±9.2
Overall (μ_1)		240 (131)	56.5±2.1	127.4±11.4	166.6±18.0	39.2±6.5
A. marmorata	Cagayan River, PH	45 (13)	49.5±1.5	95.4±14.1	131.2±15.2	35.8±8.7
	Hsiukuluan River, TW	86 (13)	51.6±1.6	104.4±12.3	127.0±15.4	22.6±6.6
	Kurio River, JP	37 (15)	46.7±1.7	109.2±16.8	137.0±17.8	27.3±8.9
Overall (μ2)		168 (41)	49.3±1.6	103.0±14.4	131.7±16.1	28.6±8.1
Difference (μ_1-μ_2)			7.2	24.4	34.9	10.6
Significance			*A. j.* > *A. m.*	*A. j.* > *A. m.*	*A. j.* > *A. m.*	*A. j.* > *A. m.*

*Cheng and Tzeng (1996)

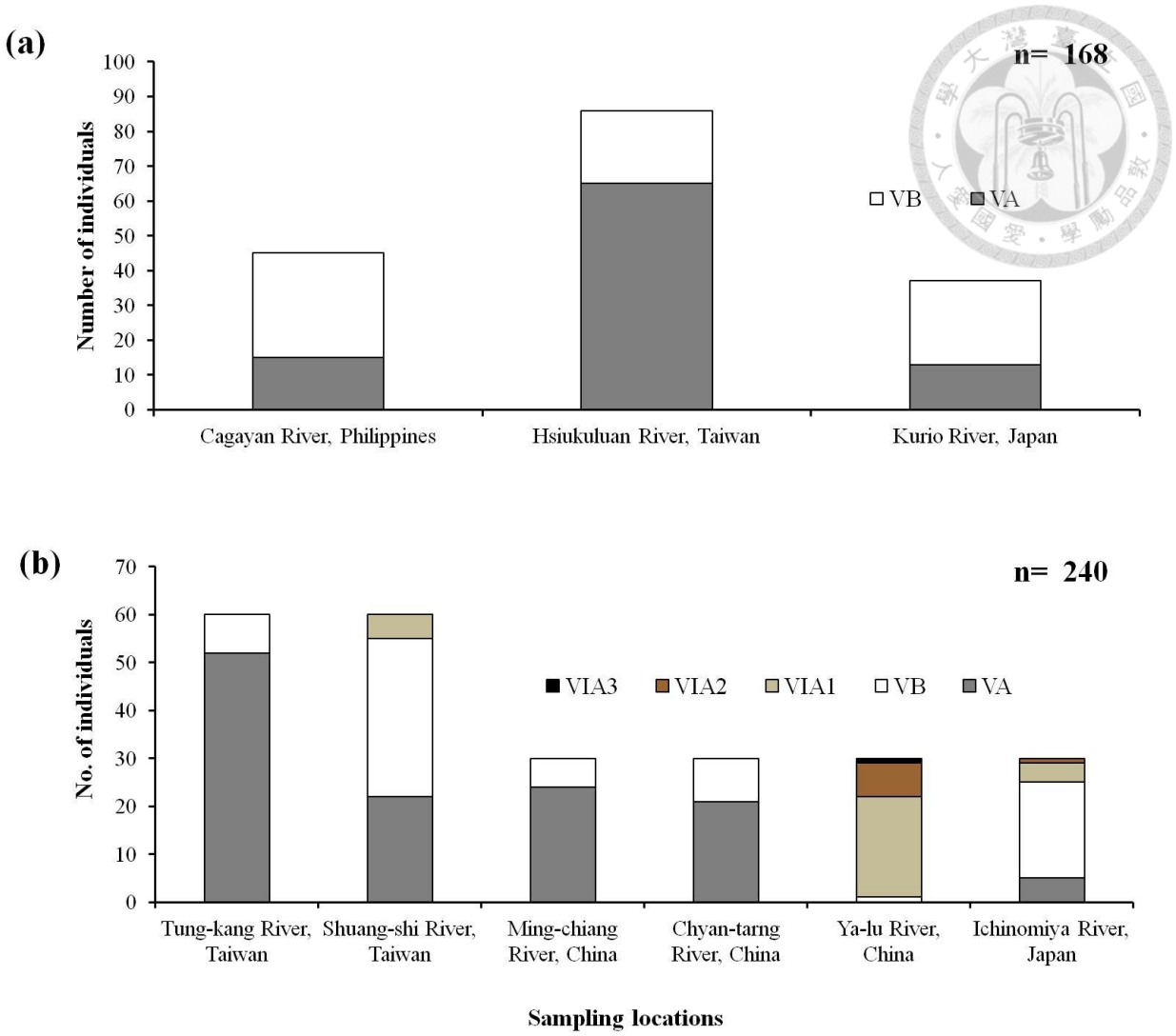


Fig. 27. Developmental stage composition of recruiting *Anguilla marmorata* (a) and *A. japonica* glass eels.

Table 8. Pigmentation stages of glass eels of *Anguilla japonica* and *A. marmorata* collected from various rivers and estuaries in East Asia.

Species	Sampling site	n	Pigmentation stage						
			V_A	V_B	VI_{A1}	VI_{A2}	VI_{A3}	VI_{A4}	VI_B
*A. japonica**	Tungkang River, Taiwan	30	28	2	0	0	0	0	0
		30	24	6	0	0	0	0	0
	Shuangshi River, Taiwan	30	13	17	0	0	0	0	0
		30	9	16	5	0	0	0	0
	Mingchiang River, China	30	24	6	0	0	0	0	0
	Chyantarng River, China	30	21	9	0	0	0	0	0
	Yalu River, China	30	0	1	21	7	1	0	0
	Ichinomiya River, Japan	30	5	20	4	1	0	0	0
	Total	240	124	77	30	8	1	0	0
	% composition		51.7	32.1	12.5	3.3	0.4	0	0
A. marmorata	Cagayan River, Philippines	45	15	30	0	0	0	0	0
	Hsiukuluan River, Taiwan	86	65	21	0	0	0	0	0
	Kurio River, Japan	37	13	24	0	0	0	0	0
	Total	168	93	75	0	0	0	0	0
	% composition		55.4	44.6	0	0	0	0	0

*Cheng and Tzeng (1996)

3.7 Differences in size and age at estuarine arrival between *Anguilla marmorata* and *A. japonica* glass eels

TLs of *A. japonica* glass eels at estuarine arrival ranged from 55.1 ± 1.9 mm in the Mingchiang River, southeastern China to 58.3 ± 11.3 mm in the Yalu River near the border of China and North Korea (Table 7), while those of *A. marmorata* ranged from 46.7 ± 1.7 mm in the Kurio River, southern Japan to 51.6 ± 1.6 mm in the Hsiukuluan River, eastern Taiwan. Within the same species, *A. japonica* glass eels from the Yalu River were significantly longer than those from other estuaries (*t*-test, $p < 0.01$), but those from other rivers showed no significant difference (*t*-test, $p > 0.05$). On the other hand, no significant difference (*t*-test, $p > 0.05$) in TL was observed among *A. marmorata* samples. The length-frequency distribution of recruiting *A. marmorata* and *A. japonica* glass eels in the Philippines, Taiwan, China, and Japan are shown in Fig. 28 and 29. *Anguilla japonica* glass eels at estuarine arrival were significantly longer than those of *A. marmorata* ($p < 0.001$). The T_t was observed to be significantly older ($p < 0.001$) in *A. japonica* (181.8 ± 16.2 d) than *A. marmorata* (141.6 ± 15.8 d), indicating that the latter were recruited to the estuary earlier than the former (Table 7). On the other hand, the duration of migration from the time of metamorphosis to the time of estuarine arrival ($T_{t\text{-}m}$) was significantly longer in *A. japonica* (41.1 ± 8.8 d) than in *A. marmorata* (28.1 ± 7.9 d) ($p < 0.001$) (Table 7). This indicated that after metamorphosing, *A. japonica* experienced a longer drifting time by coastal currents before being recruited to estuaries than did *A. marmorata*.

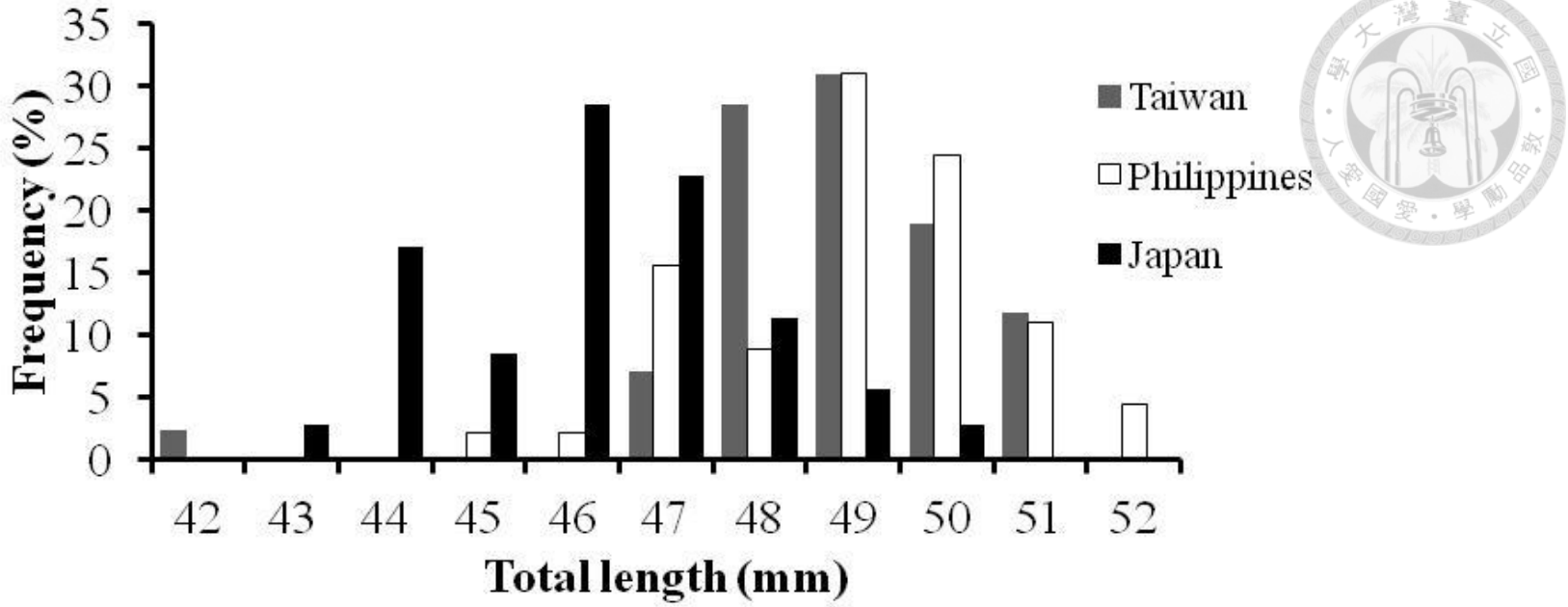


Fig. 28. Length-frequency distribution of recruiting *Anguilla marmorata* glass eels from the Philippines, Taiwan, China and Japan.

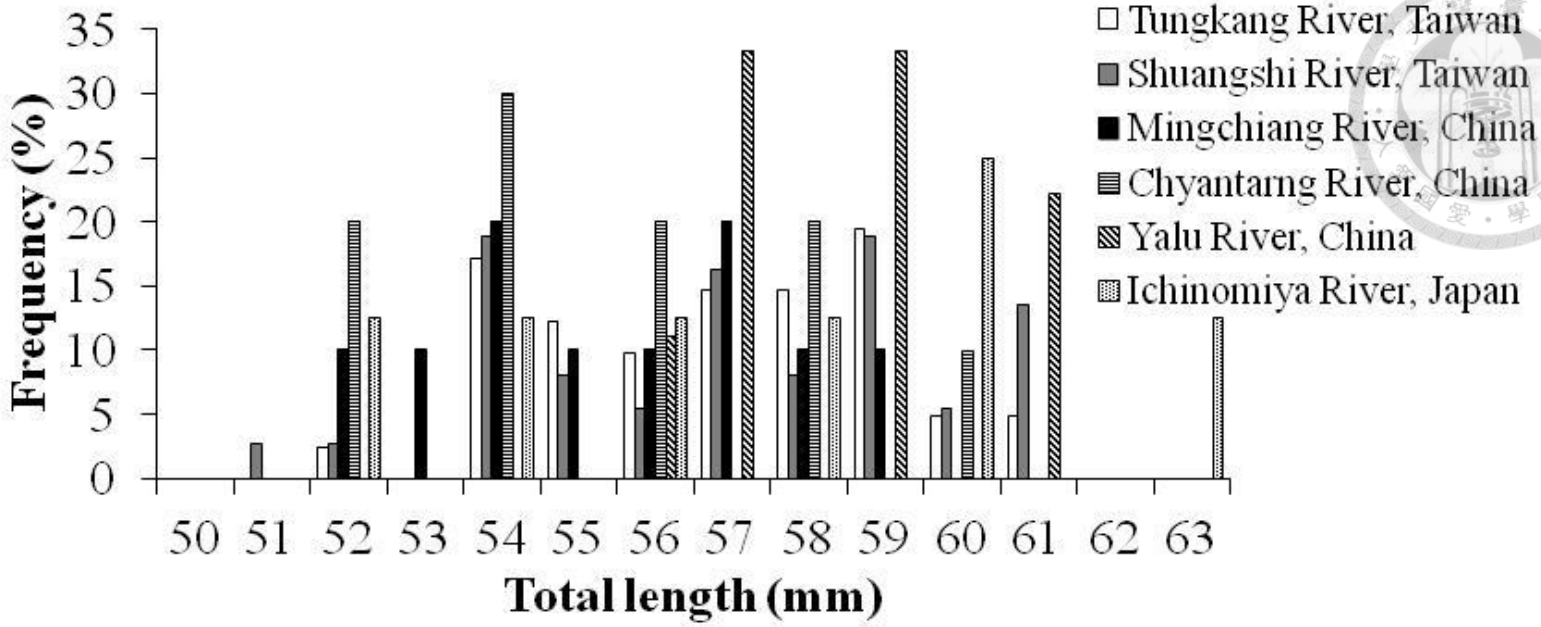


Fig. 29. Length-frequency distribution of recruiting *Anguilla japonica* glass eels from the Taiwan, China and Japan.

3.8 The timing of metamorphosis from leptocephalus to glass eel as indicated by otolith daily growth rates and Sr:Ca ratios

The overall mean otolith DGI widths of glass eels at different phases of their early life history are shown in Table 9. The mean DGI width before metamorphosis (R_m/T_m) was 0.8 ± 0.07 μm/d in *A. japonica* and 0.9 ± 0.14 μm/d in *A. marmorata*, which was narrower compared to that of the mean otolith DGI width from the metamorphosis check to the otolith edge ($R_{t\text{-}m}$) which was 1.3 ± 0.28 μm/d in *A. japonica* and 2.1 ± 0.60 μm/d in *A. marmorata*. These results indicated that otolith growth was slower during the leptocephalus stage and faster after metamorphosis in both species. From the primordium to the otolith edge, different otolith growth rates and Sr:Ca ratios were observed to correspond to different ontogenetic development stages (Fig. 30). The 1st pattern was observed in the region between the primordium and the FFC and was deposited during the yolk-sac stage. In this region, no discernible DGIs were observed, and the Sr:Ca ratio was lower because the yolk-sac was of freshwater maternal origin (Fig. 30). The 2nd pattern was observed in the region between the FFC and MC and was deposited during the leptocephalus stage. Otolith DGIs, on the other hand, became wider beyond the MC. The 3rd pattern was observed in the region between the MC and otolith edge and was deposited during the glass-eel stage. The Sr:Ca profile from the primordium to the otolith edge of the *A. marmorata* glass eels collected from the Philippines, Taiwan and Japan were shown in Figs. 31-33. These growth and Sr:Ca ratio patterns in otoliths of *A. marmorata* glass eels were similar to those observed in *A. japonica*. This indicated that both species have similar life histories from the spawning ground to the estuary in their early life stage.

Table 9. Mean (± SD) increment widths of otoliths radii R_m, R_t, and R_{t-m} in of *Anguilla japonica* and *A. marmorata* glass eels. *n*, number of individuals used for increment width measurements

Species	Sampling site	Sampling date	Increment width in μm (*n*)		
			R_m	R_t	R_{t-m}
A. japonica [a]	Tungkang River, Taiwan	30 Dec. 92	0.8 ± 0.07 (5)	0.9 ± 0.09 (5)	1.2 ± 0.31 (5)
		24 Mar. 93	0.8 ± 0.07 (7)	0.8 ± 0.03 (5)	1.4 ± 0.19 (5)
	Shuangshi River, Taiwan	20 Dec. 92	0.8 ± 0.07 (7)	0.9 ± 0.03 (6)	1.5 ± 0.48 (6)
		17 Feb. 93	0.9 ± 0.09 (3)	1.0 ± 0.04 (2)	1.4 ± 0.03 (2)
	Mingchiang River, China	1 Mar. 93	0.7 ± 0.02 (5)	0.9 ± 0.06 (5)	1.4 ± 0.38 (5)
	Chyantarng River, China	17 Feb. 93	0.7 ± 0.08 (7)	0.8 ± 0.03 (4)	1.3 ± 0.17 (4)
	Yalu River, China	3 May 93	0.8 ± 0.09 (8)	0.9 ± 0.05 (8)	1.3 ± 0.45 (8)
	Ichinomiya River, Japan	10 Jan. 94	0.7 ± 0.06 (5)	0.8 ± 0.05 (5)	1.2 ± 0.26 (5)
	Overall (μ_1)		0.78 ± 0.07 (47)	0.88 ± 0.05 (40)	1.34 ± 0.28 (40)
A. marmorata	Cagayan River, the Philippines	19 May 08	1.0 ± 0.18 (13)	1.1 ± 0.14 (13)	1.84 ± 0.46 (13)
	Hsiukuluan River, Taiwan	20 May 08	0.9 ± 0.10 (13)	1.2 ± 0.18 (13)	2.29 ± 0.72 (13)
	Kurio River, Japan	6 June 96	0.9 ± 0.14 (15)	1.1 ± 0.17 (15)	2.18 ± 0.61 (15)
	Overall (μ_2)		0.93 ± 0.14 (41)	1.13 ± 0.16 (41)	2.10 ± 0.60 (41)
Difference (μ_2-μ_1)			0.15	0.25	0.76
Significance			*A.m.* > *A.j.*	*A.m.* > *A.j.*	*A.m.* > *A.j.*

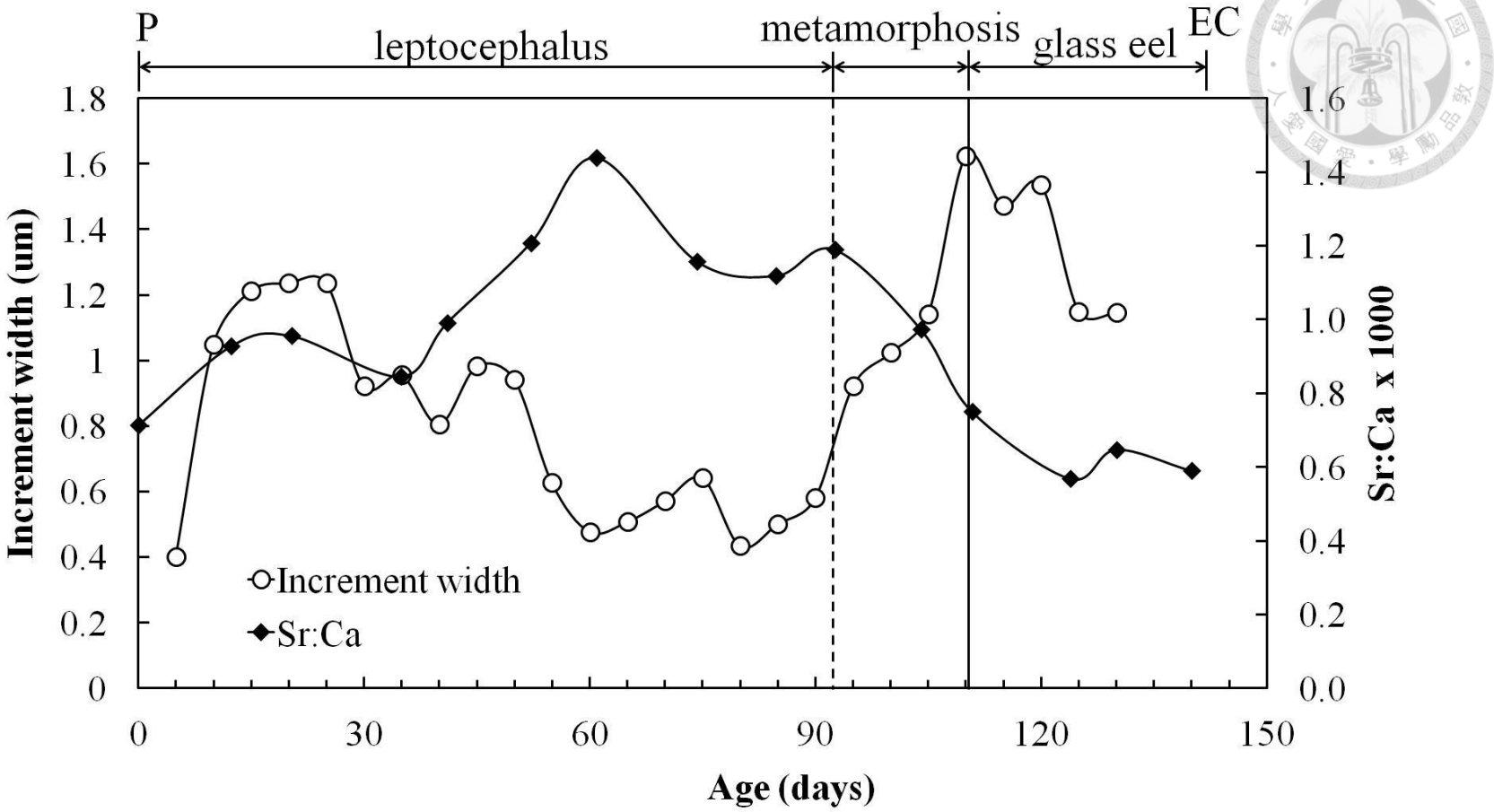


Fig. 30. Profiles of otolith growth increment width (blank circle) and Sr:Ca concentration ratios measured using electron probe microanalyzer (solid diamond) from the primordium to the edge in *A. marmorata* glass eels. The vertical dashed and solid lines indicate the age at the onset of metamorphosis and age at termination of metamorphosis, respectively. Primordium (P) and elver check (EC).

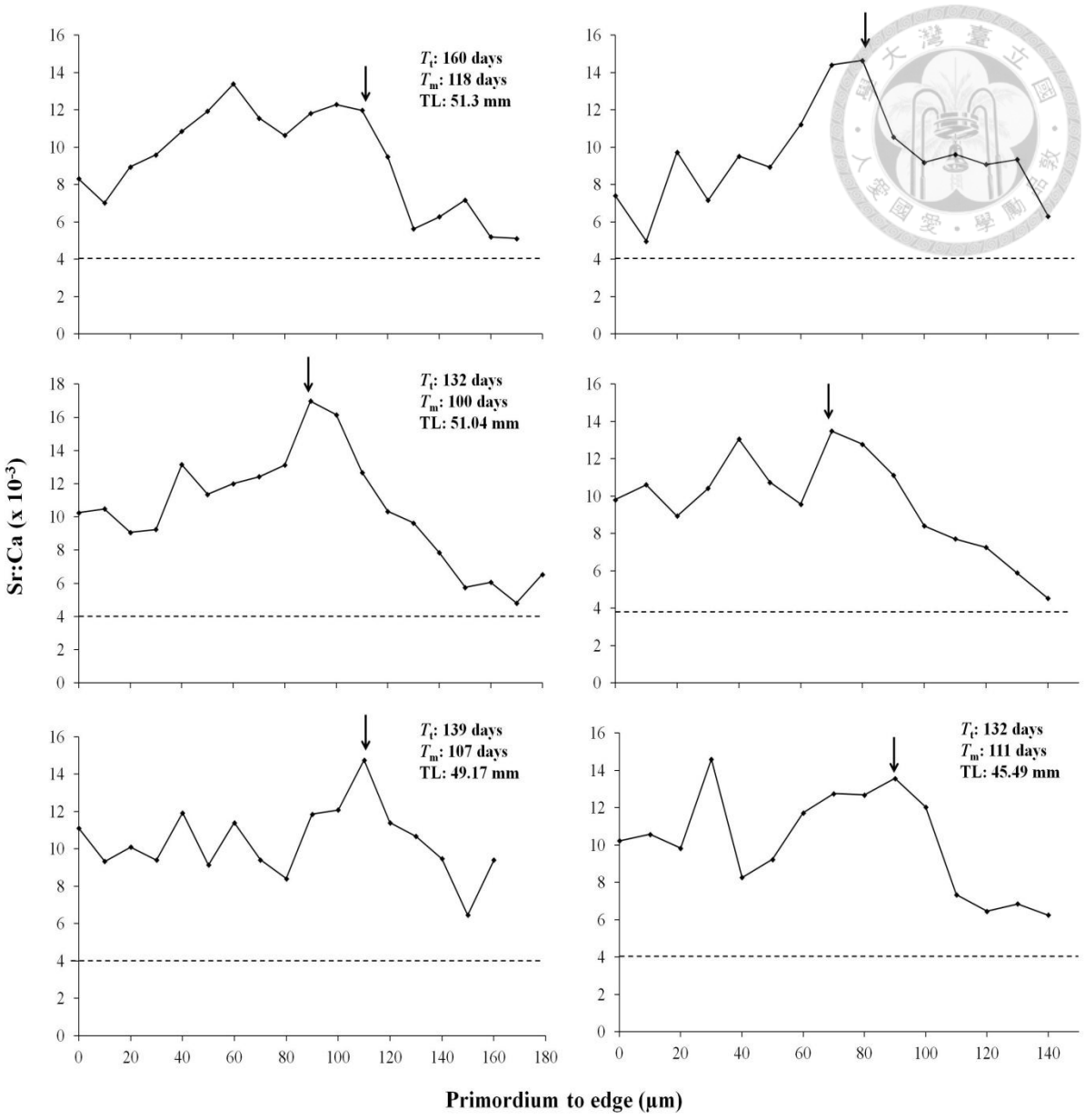


Fig. 31. Otolith Sr:Ca profiles of the *A. marmorata* glass eels collected from the Philippines. The arrows indicate the onset of metamorphosis from leptocephalus to glass eel. Dashed lines indicate the freshwater mark. When applicable, the age at estuarine arrival (T_t), age at metamorphosis (T_m) and total length at recruitment (TL) of each individual analyzed are shown in the upper right.

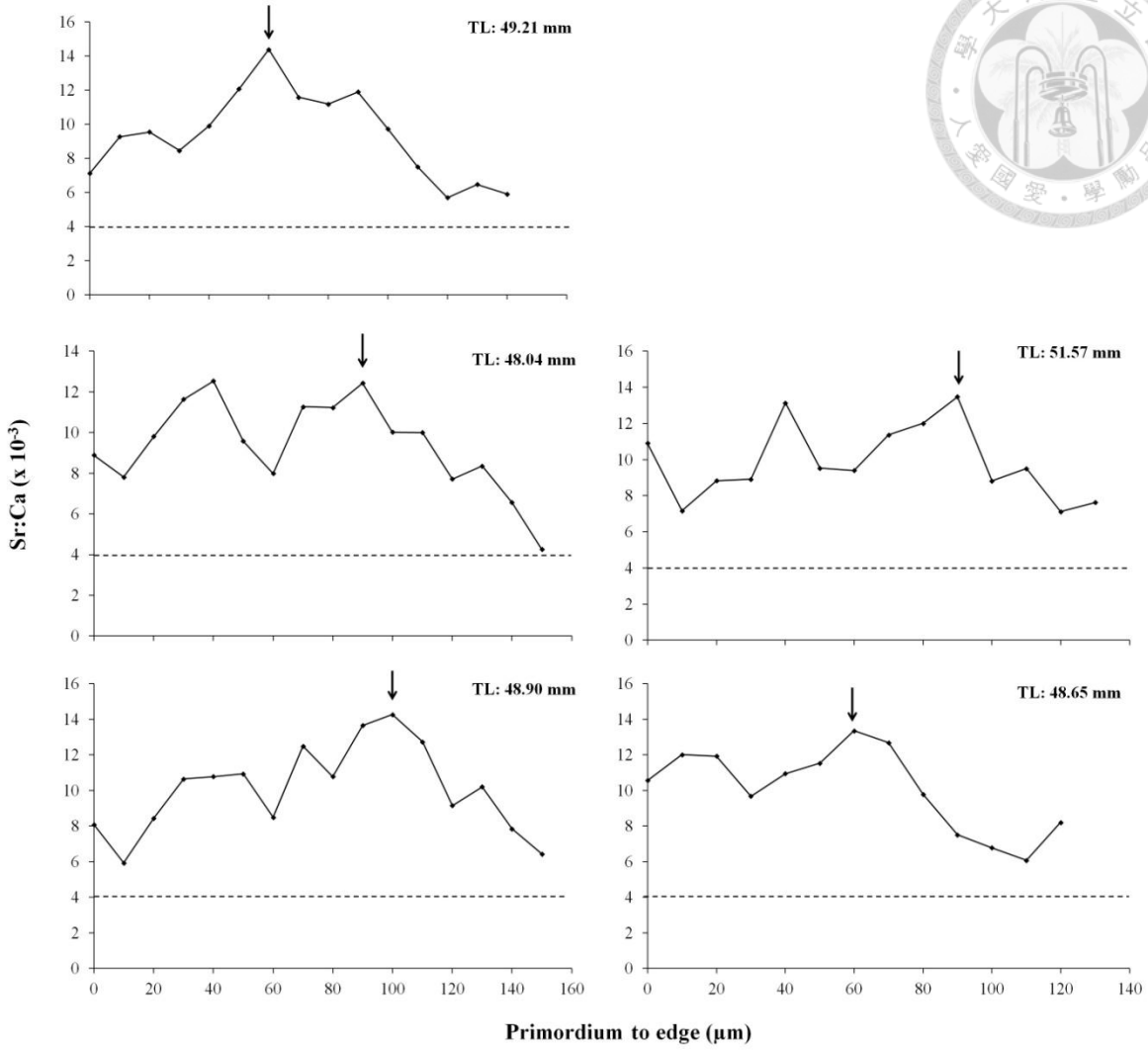


Fig. 32. Otolith Sr:Ca profiles of the *A. marmorata* glass eels collected from Taiwan. The arrows indicate the onset of metamorphosis from leptocephalus to glass eel. Dashed lines indicate the freshwater mark. Values on the upper right hand corner of each panel indicated the total length (TL) at recruitment.

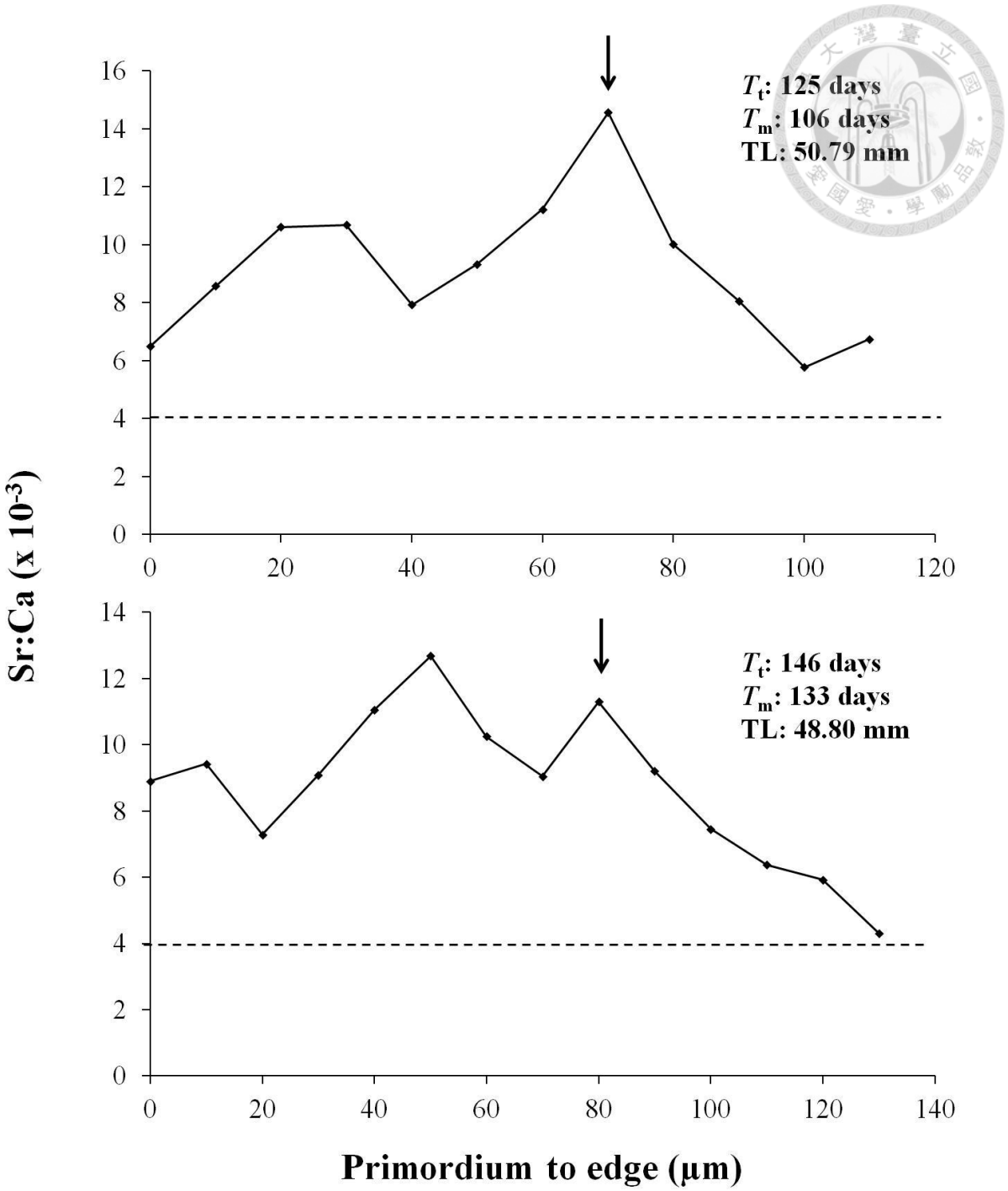


Fig. 33. Otolith Sr:Ca profiles of the *A. marmorata* glass eels collected from Japan. The arrows indicate the onset of metamorphosis from leptocephalus to glass eel. Dashed lines indicate the freshwater mark. When applicable, the age at estuarine arrival (*T*t), age at metamorphosis (*T*m) and total length at recruitment (TL) of each individual analyzed are shown in the upper right.

3.9 Back-calculated hatching dates of *Anguilla marmorata* and *A. japonica*

Table 10 shows the back-calculated hatching dates of *A. japonica* and *A. marmorata* glass eels collected from various estuaries and rivers in East Asia. *Anguilla japonica* glass eels collected in Taiwan in December 1992 were hatched between June and July 1992 while those collected between February and March 1993 were hatched between August and November 1992. Those collected from Mingchiang and Chyantarng Rivers in February to March 1993 and Yalu River in May 1993all in China were hatched between August and October of the previous year. Glass eels collected from Ichinomiya River in Japan in January 1994 were hatched in July of the previous year.

Anguilla marmorata glass eels collected from Cagayan River in northern Philippines and Hsiukuluan River in eastern Taiwan in May 2008 were found to be hatched between December 2007 and January 2008 while those collected from Kurio River in Southern Japan in June 1996 were hatched between December 1995 and February 1996.

Table 10. Age and estimated hatch date of glass eels of *Anguilla japonica* and *A. marmorata* recruiting in East Asia.

Species	Sampling site	n	Sampling date	Age at recruitment	Back-calculated hatching date
A. japonica [a]	Tung-kang River, Taiwan	16	Dec.30,1992	177.7 ± 17.8	Jun. 18, 1992 - Jul. 24, 1992
		14	Mar.24,1993	174.4 ± 17.9	Oct. 27, 1992 - Nov. 25, 1992
	Shuang-shi River, Taiwan	12	Dec.20,1992	175.0 ± 20.9	Jun. 08, 1992 - Jul. 18, 1992
		13	Feb.17,1993	174.4 ± 17.7	Aug. 09, 1992 - Sept. 14, 1992
	Ming-chiang River, China	20	Mar.01,1993	172.1 ± 14.1	Aug. 27, 1992 - Sept. 24, 1992
	Chyan-tarng River, China	23	Feb.17,1993	194.9 ± 18.6	July. 19, 1992 - Aug. 25, 1992
	Ya-lu river, China	23	May 03,1993	199.3 ± 15.6	Oct. 01, 1992 - Oct. 31, 1992
	Ichinomiya River, Japan	10	Jan.10,1994	186.6 ± 7.0	Jul. 01, 1993 - Jul. 15, 1993
A. marmorata	Cagayan River, Philippines	13	May 19,2008	144.8 ± 14.2	Dec. 05, 2007- Jan. 13, 2008
	Hsiukuluan River, Taiwan	13	May 20,2008	134.0 ± 15.4	Dec. 10, 2007 - Jan. 22, 2008
	Kurio River, Japan	15	Jun.06,1996	145.0 ± 17.8	Dec. 19, 1995 - Feb. 20, 1996

[a]Cheng and Tzeng (1996)

3.10 Age at metamorphosis in relation to the growth rate and distance from the spawning grounds

The overall mean (± standard deviation) T_m was significantly older in *A. japonica* (140.7 ± 13.6 d) than in *A. marmorata* (113.5 ± 13.0 d) ($p < 0.001$, Table 7). Spatial changes in T_m, T_t, TL and $T_{t\text{-}m}$ of *A. marmorata* and *A. japonica* were shown in Fig. 34 to 37. It was found that T_m increased from south to north in both species (Fig. 34). In addition, the T_m was negatively correlated with the growth rate before metamorphosis (G_m) for both *A. marmorata* (Fig. 38) and *A. japonica* (Fig. 39). On the other hand, T_m values of *A. marmorata* and *A. japonica* were positively related to the larval dispersal distance from the spawning grounds (Fig. 40).

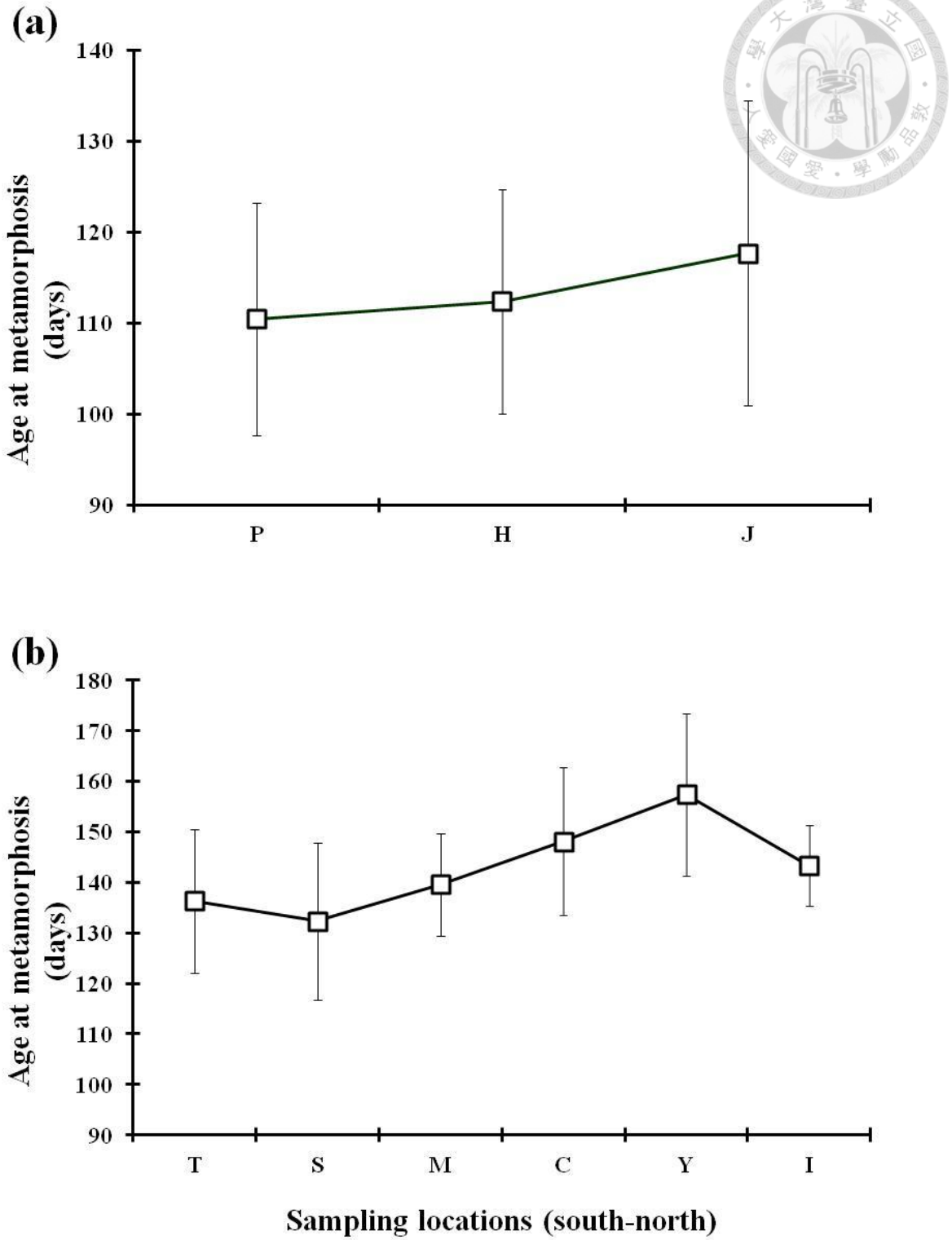


Fig. 34. Spatial changes in age at metamorphosis in *A. marmorata* (a) and *A. japonica* (b). Abbreviations of sampling locations are given in Figure 9.

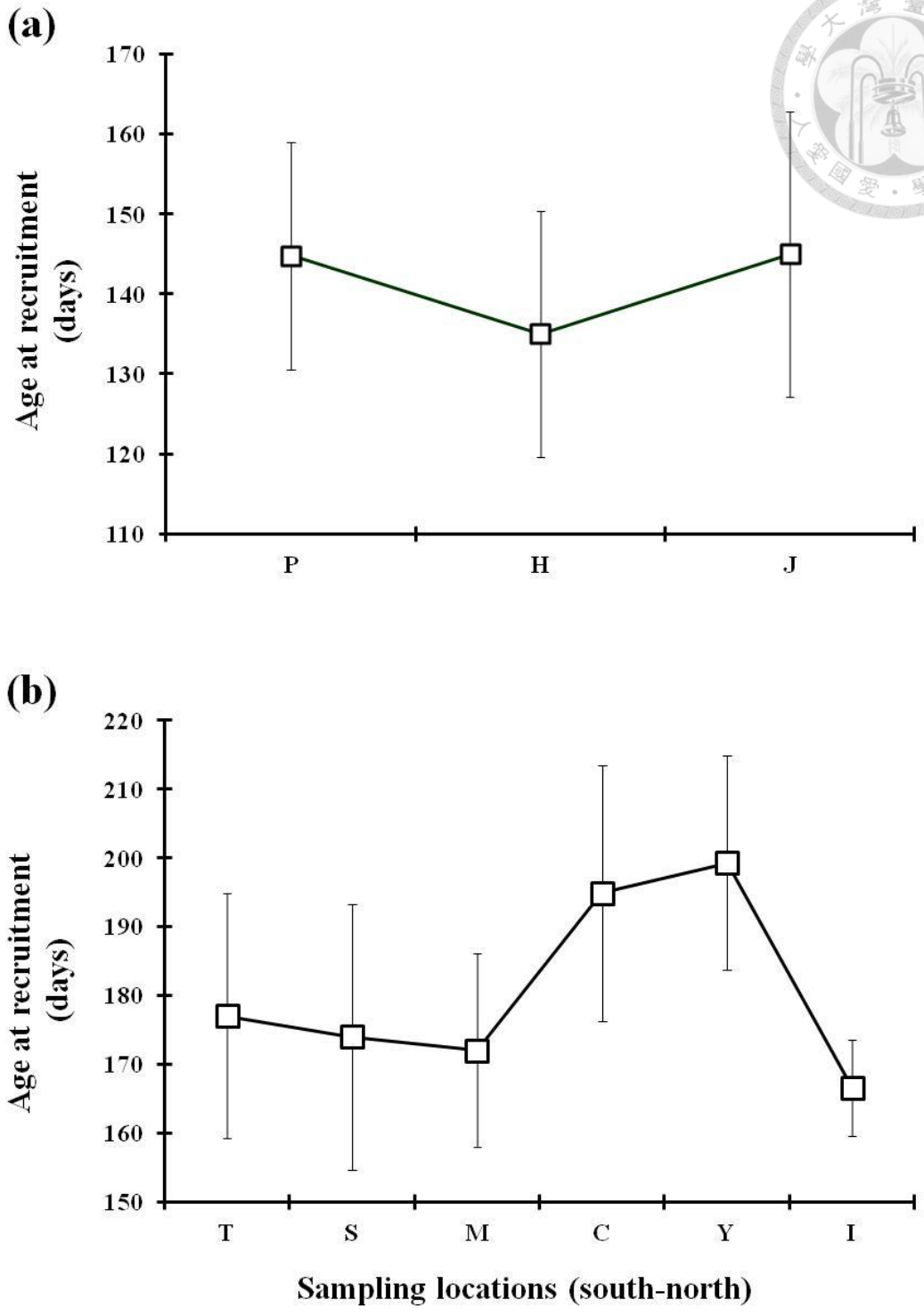


Fig. 35. Spatial changes in age at recruitment in *A. marmorata* (a) and *A. japonica* (b). Abbreviations of sampling locations are given in Figure 9.

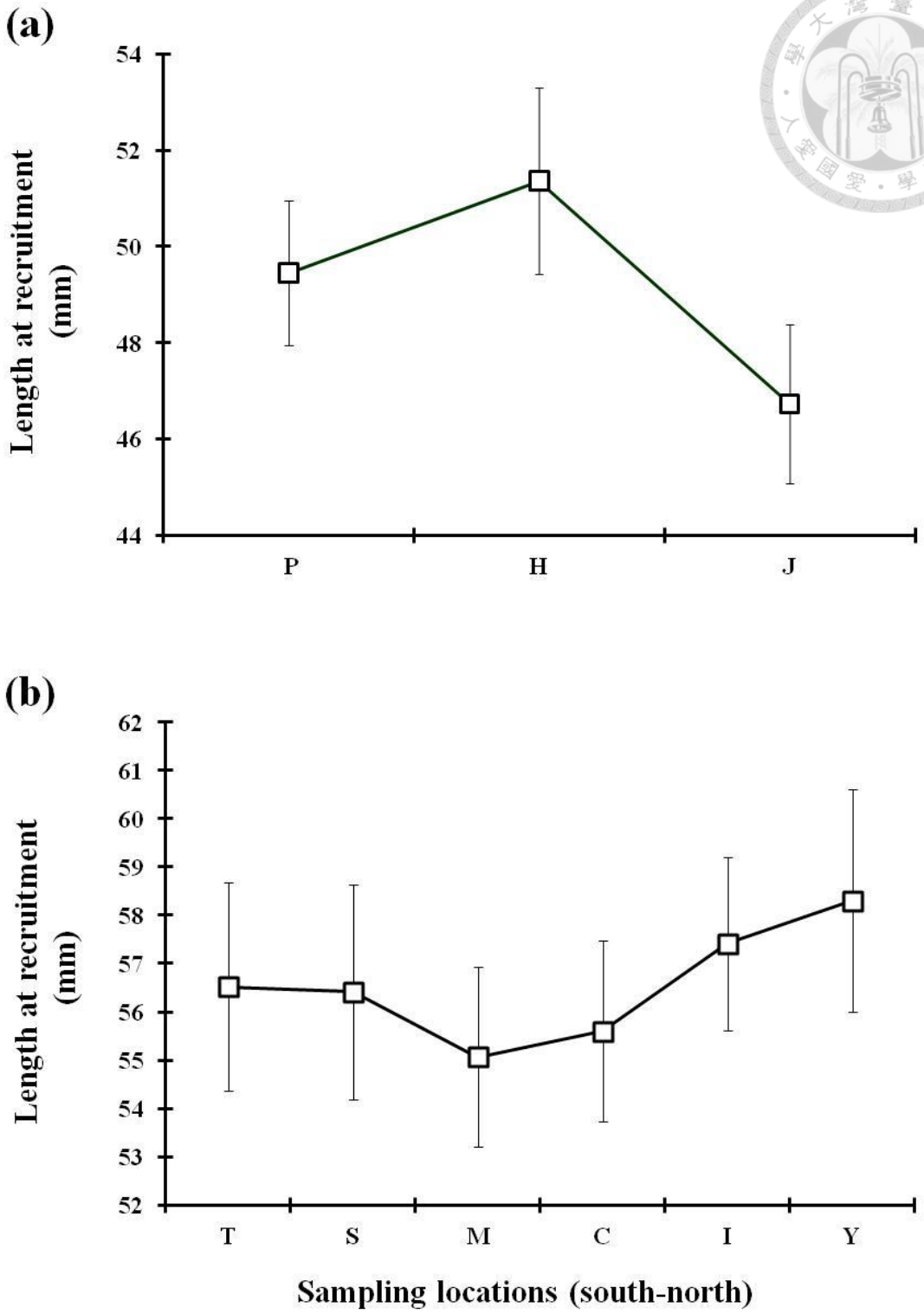


Fig. 36. Spatial changes in length at recruitment in *A. marmorata* (a) and *A. japonica* (b). Abbreviations of sampling locations are given in Figure 9.

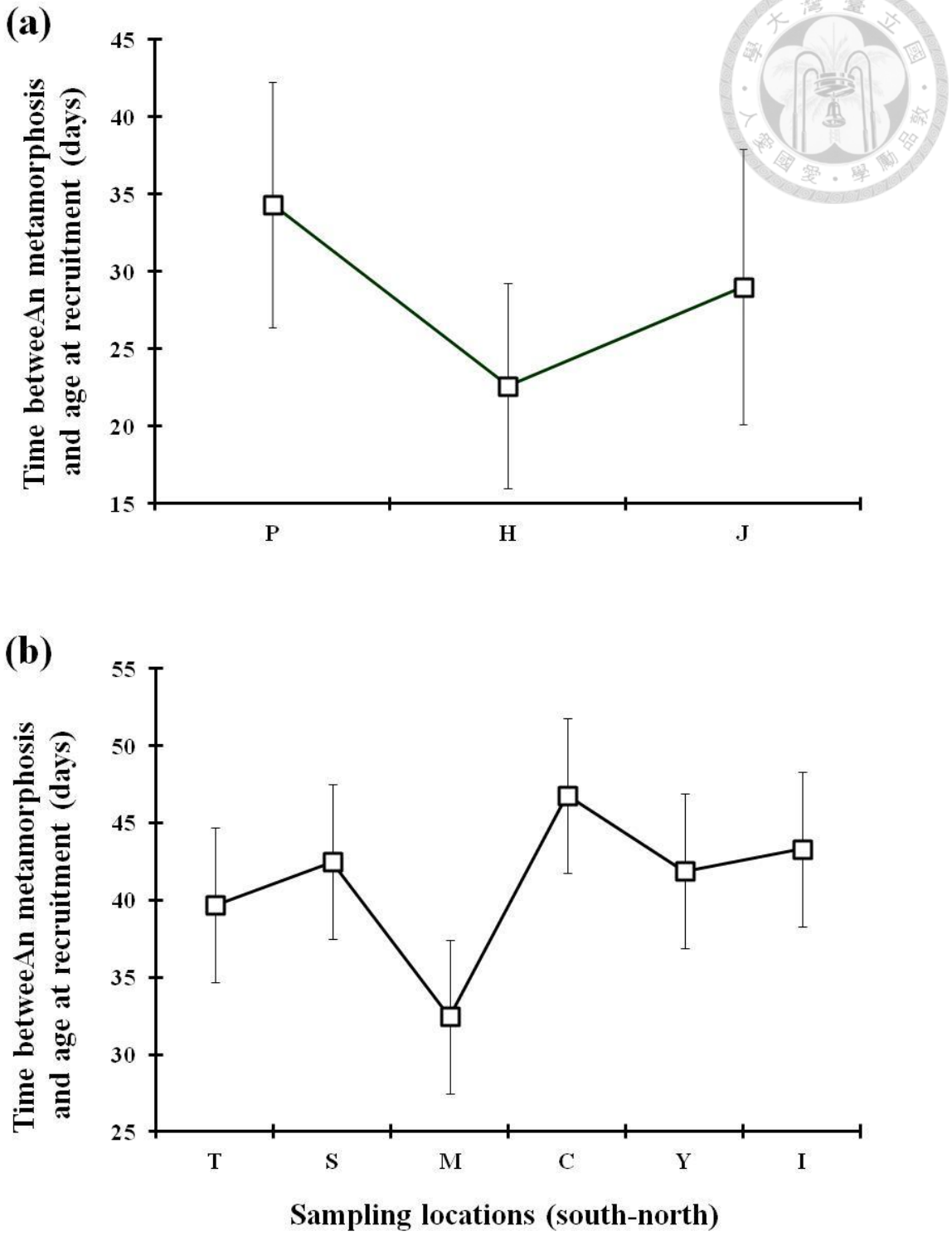


Fig. 37. Spatial changes in time between metamorphosis and age at recruitment in *A. marmorata* (a) and *A. japonica* (b). Abbreviations of sampling locations are given in Figure 9.

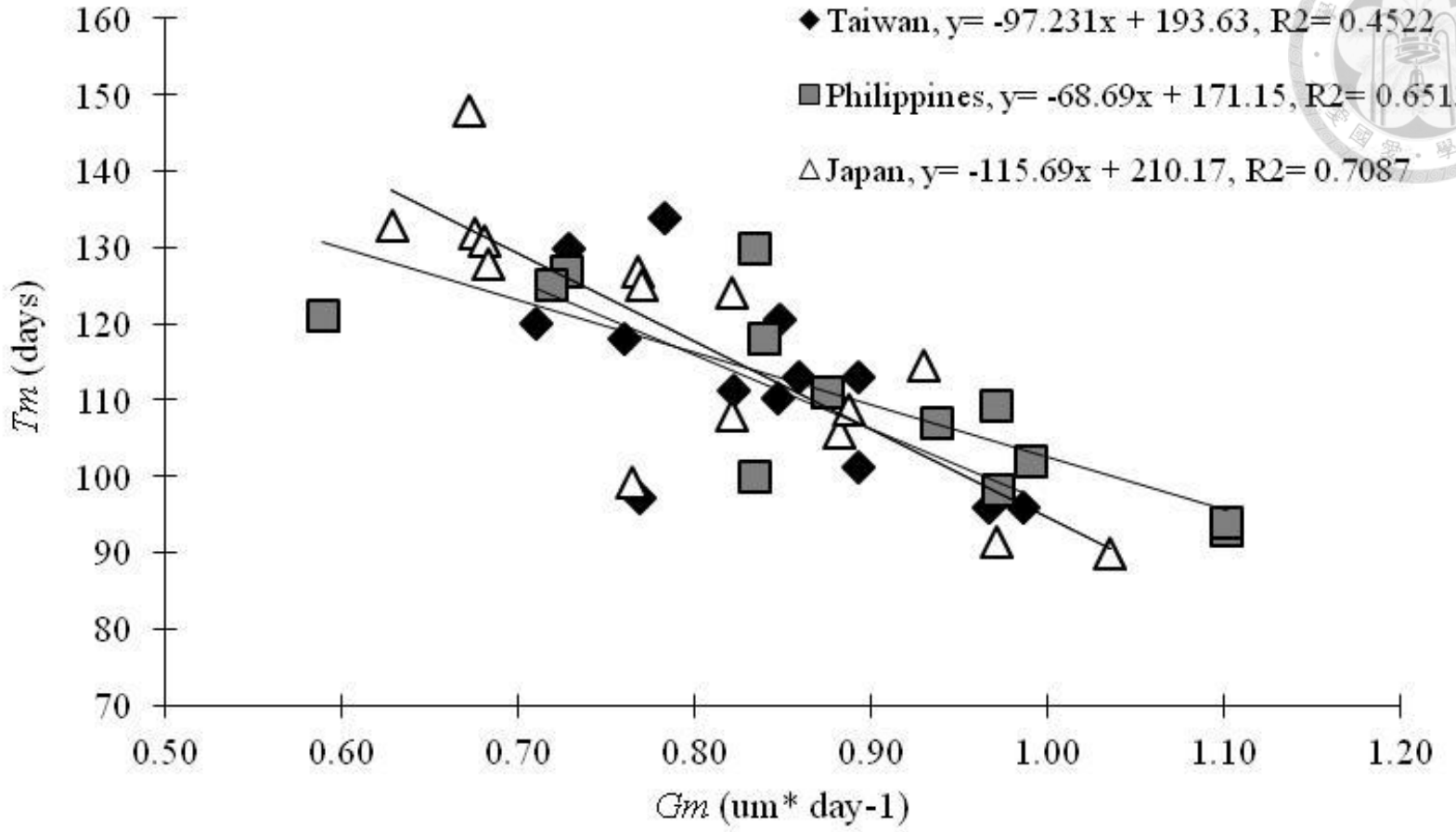


Fig 38. Relationship between the age at metamorphosis (T_m) and growth rate (G_m) before metamorphosis in *Anguilla marmorata*.

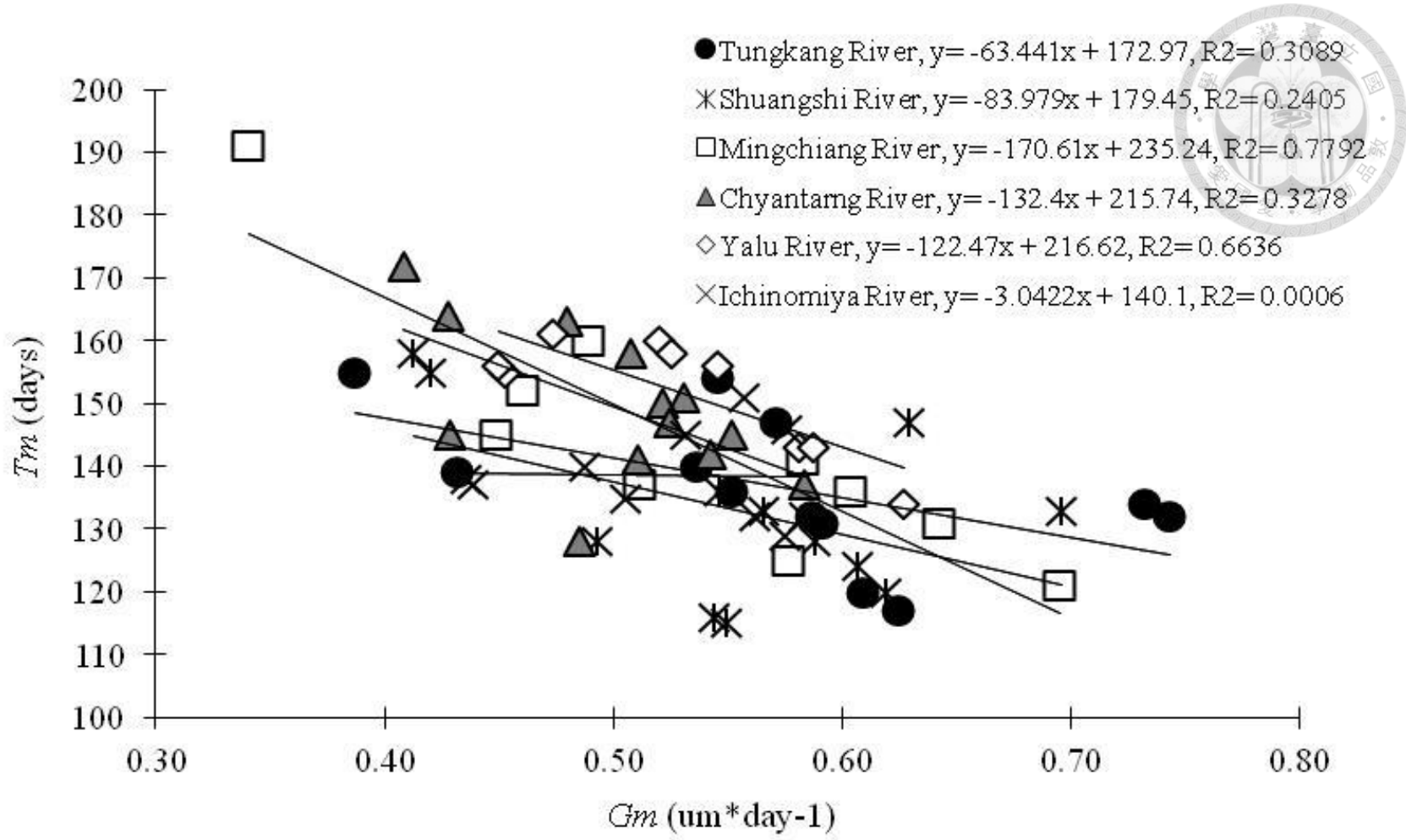


Fig 39. Relationship between the age at metamorphosis (T_m) and growth rate (G_m) before metamorphosis in *Anguilla japonica*.

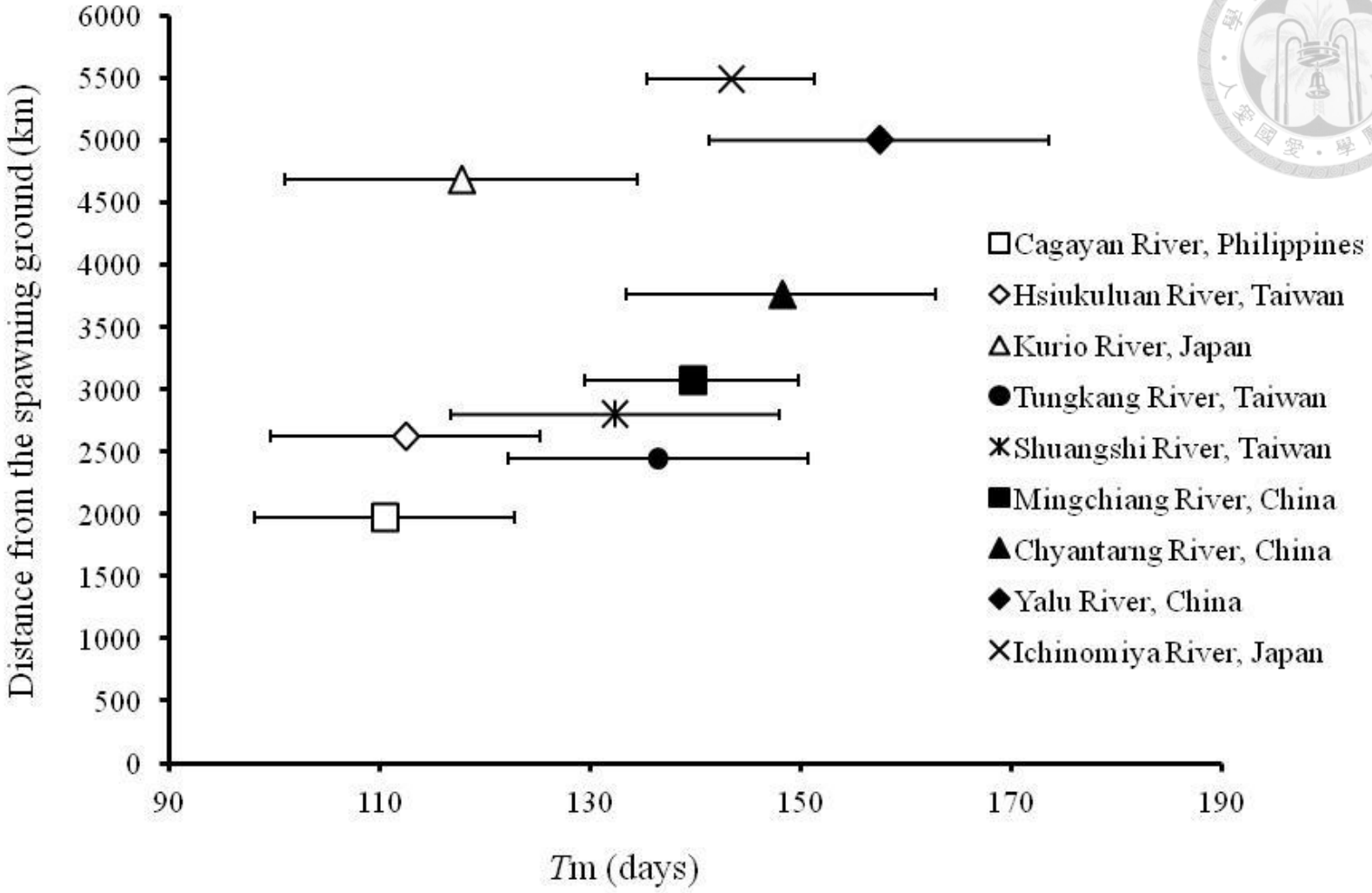


Fig 40. Relationship between the larval dispersal distance and age at metamorphosis (T_m) in *Anguilla marmorata* (open symbols) and *A. japonica* (solid symbols).

3.11 Migratory environmental history and habitat use of juvenile *Anguilla marmorata* in the Philippines as indicated by otolith Sr:Ca ratios

A summary of total length, weight, sex, otolith length and weight and age of *A. marmorata* samples analyzed in this study were shown in Tables 11 and 12. The majority of the samples from Hainan were female while the samples from the Philippines were unsexed. The TL of *A. marmorata* samples from the Philippines ranges from 344.06 to 553.86 mm (Table 11) while that of the samples from Hainan ranges from 770 to 975 mm (Table 12). On the other hand, the average age of the samples from the Philippines was 2.429±0.54 while that of the samples from Hainan was 3.36±0.50. Samples of the otoliths of wild and cultured *A. marmorata* were shown in Figs. 41 and 42.

Table 11. Sampling information of the adult eels collected from a river system in Aurora province, eastern Luzon, Philippines on August 6, 2012. OTW: otolith weight, OTL: otolith length.

TL (mm)	OTW (g)	OTL (mm)	Age
541.13	0.0084	3.52	3
553.86	0.0059	3.67	3
553.73	0.0071	3.53	3
391.33	0.0032	2.49	2
378.06	0.0029	2.38	2
344.06	0.0032	2.47	2
392.60	0.0049	2.52	2
450.681±93.971	0.005±0.002	2.940±0.596	2.429±0.535

Table 12. Sampling information of the adult eels collected from an aquaculture farm in Sanya, southern Hainan province, China. OTW: otolith weight, OTL: otolith length, n/a: not available

Sampling Date	TL (mm)	Weight(g)	Sex	OTW left(g)	OTW right(g)	OTL(mm)	Age
13-Mar-09	931	2995	F	0.0116	0.0122	4.94	3
	975	3115	F	0.0096	0.0094	4.29	3
	873	2515	F	0.0106	0.0115	4.68	n/a
	912	1940	F	0.0114	0.011	4.23	4
27-Mar-09	920	2785	F	0.0101	0.0101	4.27	n/a
	919	2455	F	0.0115	0.0123	5.13	3
	913	1950	F	0.0089	0.009	4.13	n/a
	926	1805	F	0.0114	0.0112	4.45	n/a
	860	1750	F	0.0099	0.0099	4.82	3
	830	1575	F	0.0121	0.0117	4.74	3
	858	1445	F	0.0098	0.01	4.68	4
	791	1350	F	0.0107	0.011	4.38	3
	776	1325	F	0.0093	0.0103	4.58	3
28-Mar-09	775	1385	F	0.0082	0.0091	4.32	n/a
	794	1185	F	0.0126	0.0126	4.45	4
	785	1145	F	0.0092	0.009	4.76	4
	770	1230	F	0.0096	0.0098	4.37	n/a
Mean±S.D.	859.29±67.733	1878.41±657.386		0.010±0.001	0.011±0.001	4.542±0.278	3.36±0.504

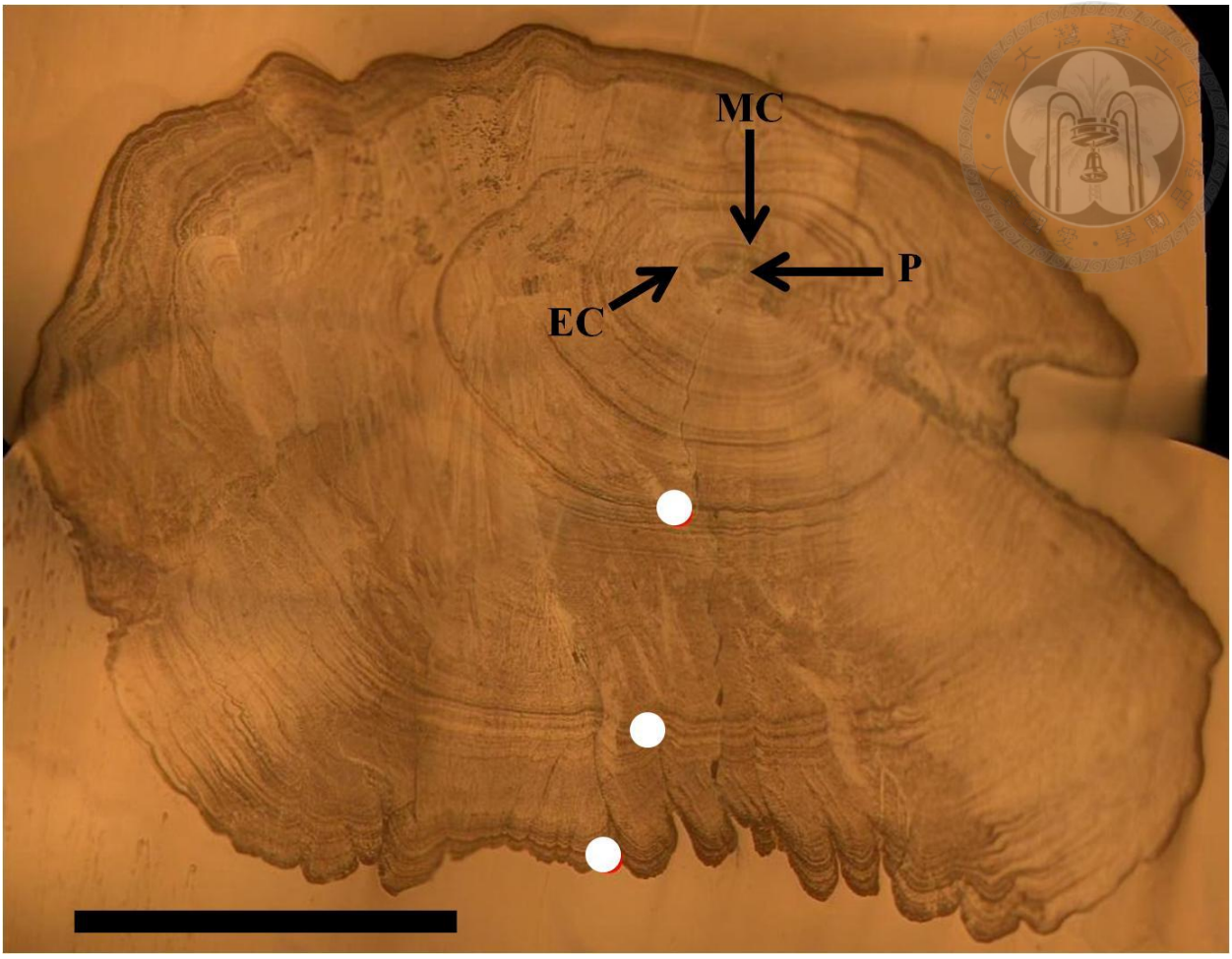


Fig. 41. An otolith sample of a wild *Anguilla marmorata* (553.86 mm TL) from Aurora province, Philippines. White circles: annulus; P: primordium; MC: metamorphosis check and EC: elver check. Scale bar= 500 µm.

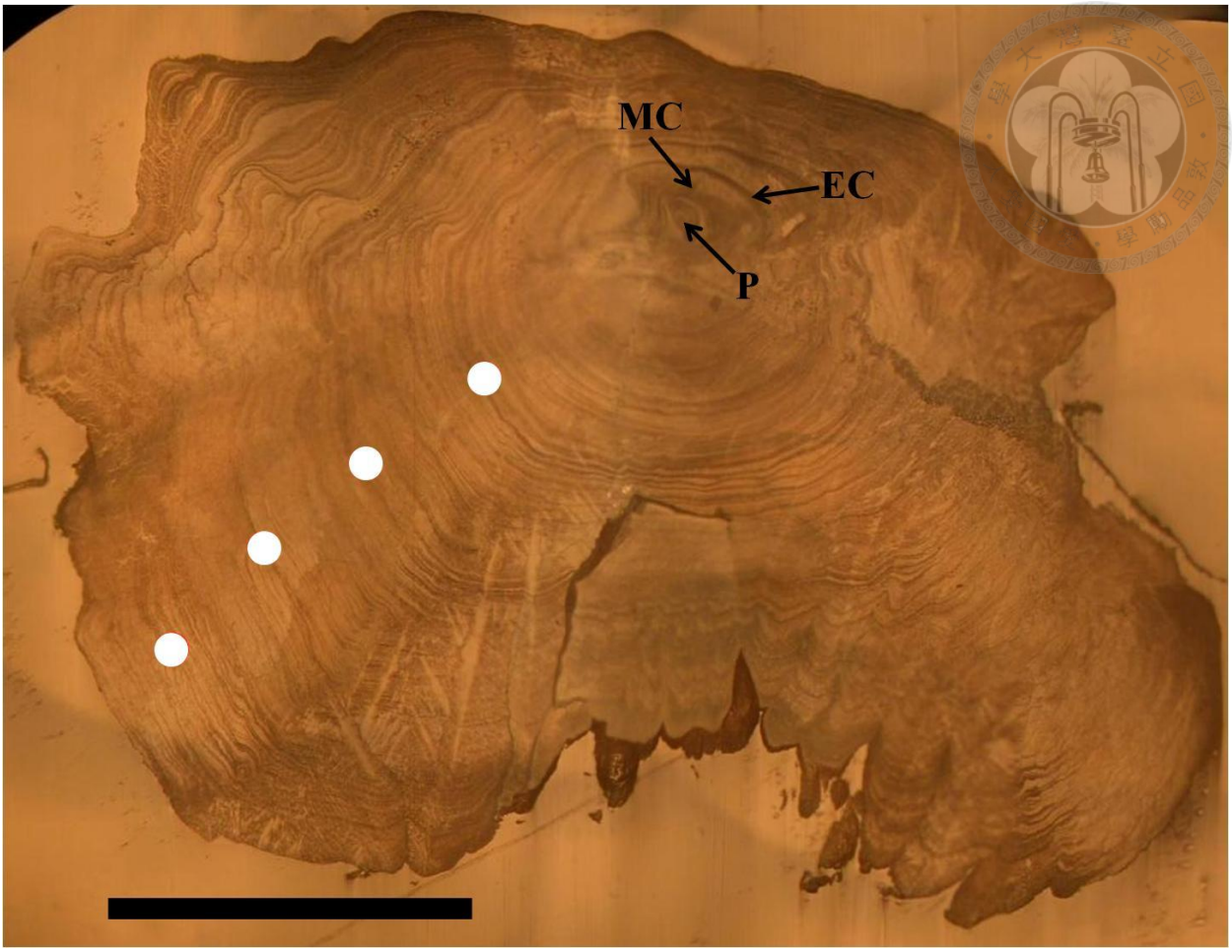


Fig. 42. An otolith sample of a cultured *Anguilla marmorata* (785 mm TL) from Hainan province, China. White circles: annulus; P: primordium; MC: metamorphosis check and EC: elver check. Scale bar= 500 μm.

In all the otoliths examined (wild and aquacultured), each had a high Sr:Ca ratio in the central region that corresponded to the marine leptocephalus stage up to the early glass eel stage during the part of the life history from their spawning grounds to the coastal waters and showed lower Sr:Ca ratio levels ($< 4 \times 10^{-3}$) (Fig. 43-44) as they migrate to the brackishwater. Each otolith had a peak Sr:Ca ratio that ranges from 10.1×10^{-3} - 13.6×10^{-3} in the otolith core. The ratios in the otoliths of the eels before the elver stage were similar among specimens, indicating that the migratory history was similar among specimens during the oceanic leptocephalus stage. Beyond the elver check, different otolith Sr:Ca ratio was observed between the samples collected from the wild and from aquaculture farm. The otolith Sr:Ca ratios after the elver check of the samples from the wild maintained a value $< 4 \times 10^{-3}$ until the otolith edge.

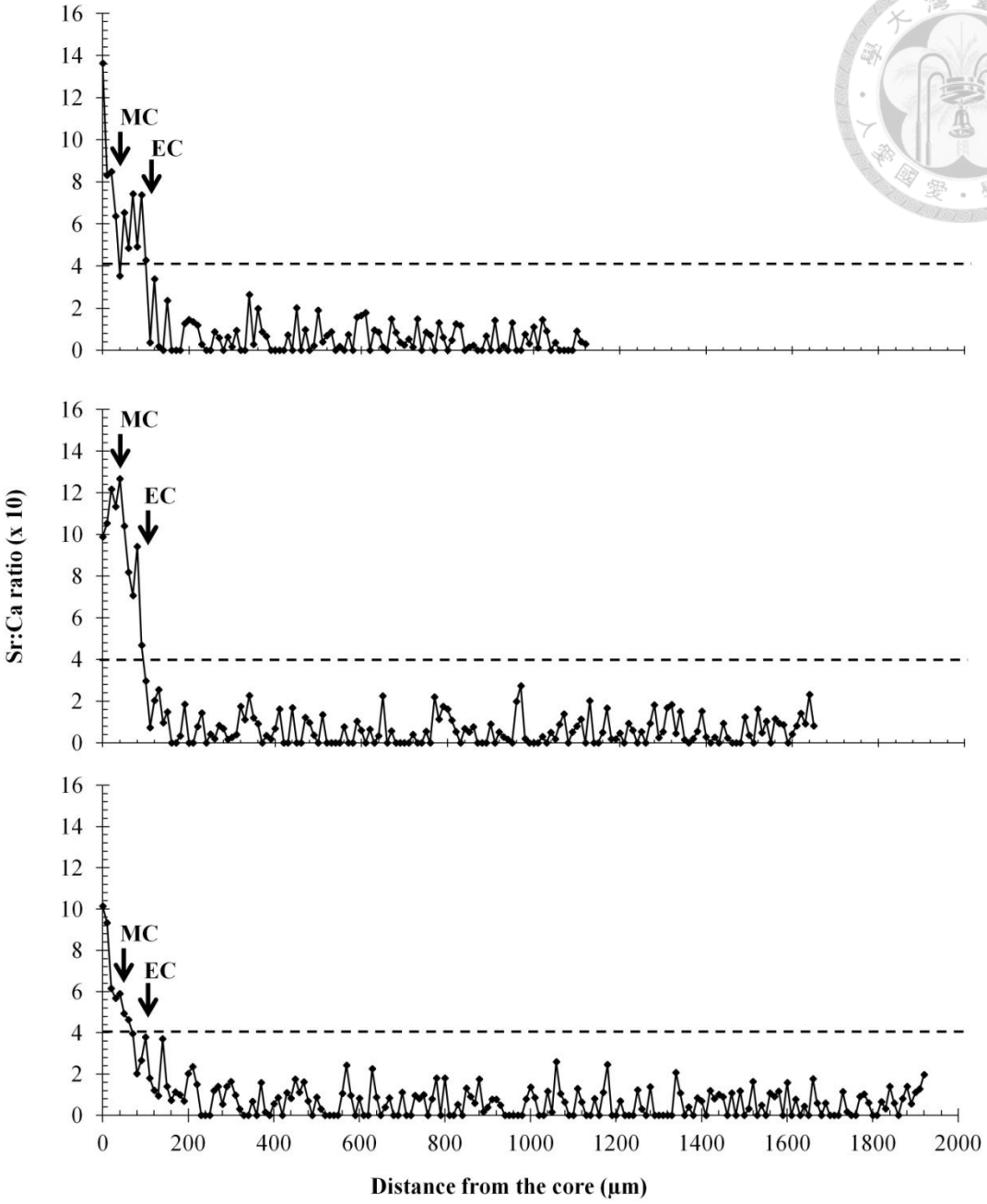


Fig. 43. Temporal changes in the otolith Sr:Ca ratios (measured from the core to the edge) of *A. marmorata* collected from the Philippines. Dashed line indicates the freshwater mark. MC: metamorphosis check; EC: elver check.

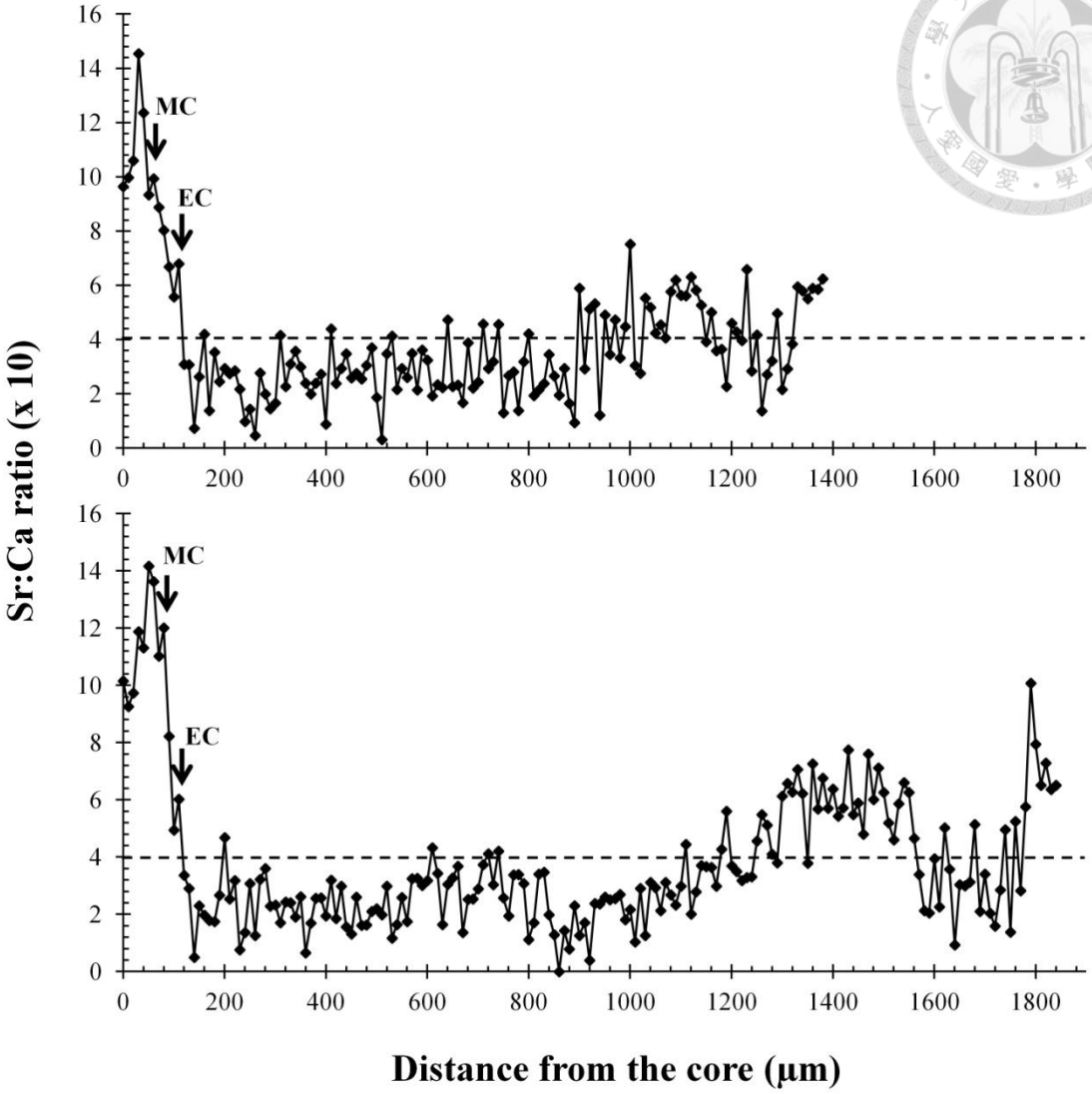


Fig. 44. Temporal changes in the otolith Sr:Ca ratios (measured from the core to the edge) of *A. marmorata* collected from an aquaculture farm in Sanya, southern Hainan Province, China. Dashed line indicates the freshwater mark. MC: metamorphosis check; EC: elver check.

4. DISCUSSION

4.1 The differences in spatial recruitment patterns among species of glass eel in Taiwan

The species composition of glass eels recruiting to the Hsiukuluan River, eastern Taiwan, was dominated by *A. marmorata* which greatly differs from that reported from rivers in southern, western, and northern Taiwan where *A. japonica* dominates (data from Tzeng 1982, 1983b; Tzeng and Chang 2001; Fig. 45). The low relative abundance of *A. japonica* in this study was doubtful at first, because it might have been due to sampling bias due to fisherman sorting out *A. japonica* for aquaculture before handing the glass eel samples to the researchers. But a recent study by Han et al. (unpublished data) in the same river system also revealed that the relative abundance of *A. japonica* was very low (< 1%). But why does the dominant species of recruiting glass eels differ between the east and west coasts of Taiwan? This scenario can be further explained by the different geographical distributions, temperature preferences, and habits of the different eel species. During the leptocephalus stage, *A. marmorata* and *A. japonica* might not have different distributions because they use the same spawning ground to the west of Mariana Island, and their larvae are transported by the NEC and Kuroshio Current to their destinations (Kuroki et al. 2009, Miller et al. 2009). But after metamorphosing from leptocephalus to glass eels, the temperate *A. japonica* migrates with the cold China Coastal Current to the west coast of Taiwan (Cheng and Tzeng 1996), while the tropical species, *A. marmorata*, *A. bicolor pacifica*, and the newly described *A. luzonensis* prefer the east coast which is influenced by the warm Kuroshio Current. Previous studies also revealed that *A. japonica* elvers were more abundant on the northern, western, and southern coasts of Taiwan than the east coast (Tzeng 1996, Tzeng and Chang 2001). Those reports indicated that species-specific differences in geographical distribution of glass eels were closely correlated to the coastal current systems which differ

between the east and west coasts of Taiwan (Tzeng 1996). Also, the species-specific recruitment, abundance, and distribution of *A. marmorata* and *A. japonica* in Taiwan can also be explained by differences in the duration of their leptocephalus stage, age at metamorphosis, and growth rate. The somatic growth rate is faster and the age at metamorphosis is younger in *A. marmorata* than *A. japonica*. Thus, the former can recruit earlier at a younger age. This must be the reason why *A. marmorata* can recruit abundantly in northern Luzon, the Philippines and along the east coast of Taiwan, while very few *A. japonica* are known to recruit there, because the latter is still in the leptocephalus stage and drifting with the Kuroshio Current. The drifting *A. japonica* leptocephali metamorphose into glass eels beyond Taiwan, and some of them then enter the westward branch of the Kuroshio Current that takes them to continental waters of East Asia where they migrate with the cold, southerly flowing China Coastal Current to the northern, western, and southern coasts of Taiwan. This scenario was validated by the peak catch of elvers that coincided with the period of the lowest winter temperatures when the northeastern monsoon-driven China Coastal Current was strongest (Tzeng 1985) and from the daily ages of elvers arriving at estuaries along the west coast of Taiwan being older in the south than in the north (Cheng and Tzeng 1996). In addition to this, Tzeng and Chang (2001) also suggested that the comparatively abundant freshwater discharges and wider shelf area along the west coast of Taiwan can potentially attract elvers to migrate upstream, unlike on the east coast where conditions might be less attractive for inshore migration and recruitment of elvers because the warm Kuroshio is very close to the shore, the salinity is higher, and the continental shelf is narrower. Compared to *A. marmorata*, the abundance of *A. bicolor pacifica* was very low, such that they can be considered an occasional species. This is because the origin or the spawning ground of these eel species is far from Taiwan. Also, the habitat use of the adult *A. japonica* and

A. marmorata living sympatrically in a river also differs (Shiao et al. 2003) with the former usually occupying lower reaches of the river while the latter occupies upper reaches.

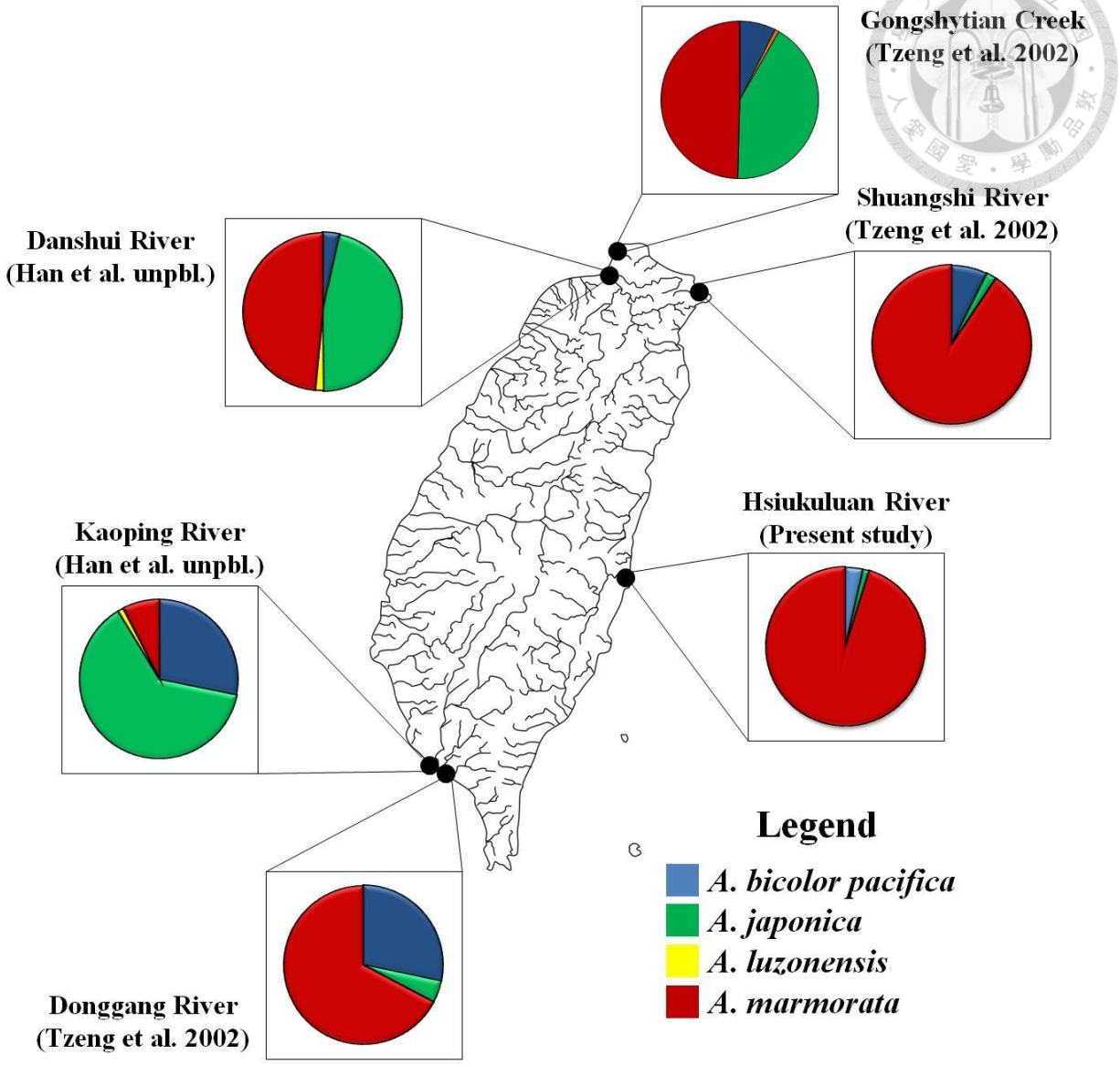


Fig. 45. Distribution patterns and abundances of *A. marmorata* and other anguillid eels in Taiwan.

4.2 Differences in seasonal occurrence between *Anguilla marmorata* and *A. japonica* in Taiwan

Previous studies indicated that the peak catch of *A. japonica* elvers in Taiwan occurred during winter in Nov. to Feb. (Tzeng 1983b, 1996, Tzeng et al. 1995), while the peak catch of *A. marmorata* elvers in the Hsiukuluan River in eastern Taiwan occurred mainly during spring and summer (Lin 2001, Han et al. unpubl. data). This difference in the recruitment season also supports different temperature preferences of these 2 species.

Differences in seasonal occurrence of the different glass eel species might be due to differences in their spawning season, temperature preference and geographic distribution. In temperate eel species, spawning occurs over a limited period, i.e., Feb. to Apr. in *A. rostrata* (McCleave et al. 1987), Mar. to June in *A. anguilla* (McCleave and Kleckner 1987), Apr. to Nov. in *A. japonica* (Tsukamoto 1990), Aug. to Dec. in *A. dieffenbachi* (Jellyman 1987), and Sept. to Feb. in *A. australis* (Jellyman 1987). With these limited spawning periods, recruitment of their glass eels is therefore limited to certain seasons. On the other hand, tropical eel species have a spawning season that persists almost throughout the year, and this year-round spawning behavior may extend the period of recruitment of their glass eels to estuarine habitats to year round, as was described in previous studies (Tabeta et al. 1976, Arai et al. 1999a,b, 2001, Shen and Tzeng 2007). In addition, fluctuations in daily catches of glass eels in estuaries are greatly influenced by the spawning duration, oceanic currents and the differences in early life history traits such as the age at metamorphosis and age at recruitment, as well as environmental cues such as the moon phase, tidal currents, and water temperature (Tzeng 1985, Cheng and Tzeng 1996, Wang and Tzeng 1998 2000).

4.3 The metamorphosis timing differs between *Anguilla marmorata* and *A. japonica* and its ecological evolution significance

In the present study, it was found that at an age of 110 d (Table 7), *A. marmorata* had already metamorphosed and commenced migration to coastal waters of the northern Philippines, while *A. japonica* remained at the pelagic leptocephalus stage and continued to drift with currents in the open ocean until it reached northern Taiwan where it began to metamorphose approximately 24 d later. This must be the reason why the geographic distribution of *A. japonica* is more northerly than that of *A. marmorata*. The metamorphosis of leptocephalus to glass eels transforms the laterally compressed, willow-leaf-like shape of the former to a more-rounded, streamlined shape of the latter (Fig. 46). This transformation reportedly causes drastic reductions in the length and weight of the leptocephalus and an estimated 80% drop in whole-body water (Bertin 1951, Otake 2003). Previous studies found that the body shape of the leptocephalus is suitable for drifting with oceanic currents (Miller 2009, Tsukamoto et al. 2009, 2011). Also, the laterally compressed willow-leaf-like body shape of the anguillid leptocephalus and the high body water content greatly contribute to its buoyancy and is favorable for passive planktonic drift and transport by ocean currents, while the body of the glass eel is more adapted for bottom dwelling. Once the leptocephalus metamorphoses into a glass eel, it loses buoyancy and leaves the strong ocean currents. In other words, metamorphosis from a leptocephalus to a glass eel in anguillid species terminates the passive drift of eel larvae and initiates migration to coastal waters, and it also determines the ultimate destination of larval dispersal. The completion of eel larval metamorphosis and the onset of the juvenile stage initiate a behavioral shift from pelagic migration to bottom settlement (Moran 1994). Earlier-metamorphosing leptocephali are recruited earlier while, delayed-metamorphosis leptocephali are bound for longer oceanic

dispersal and later estuarine recruitment. Metamorphosis occurs during migration from their offshore marine spawning grounds to their continental freshwater growth habitats, and it marks an adaptive shift from oceanic drifting to river colonization and the beginning of the continental dispersal phase (Edeline et al. 2009). DGIs in otoliths can conveniently provide the timing for metamorphosis (T_m), and the radius from the P to the MC can provide information on the "metamorphosing size" of anguillid eels. These allowed us to gain insights into the mechanism of metamorphosis of anguillid eels in the wild and provided clues to understanding the biological significance of differences in the T_m, size at metamorphosis and in growth rate of leptocephali between *A. japonica* and *A. marmorata*. Tsukamoto (1990) suggested that *A. japonica* begins to metamorphose when leptocephali reach 60 mm TL. On the other hand, metamorphosis of leptocephali of *A. marmorata* and other tropical eel species like *A. bicolor pacifica*, *A. borneensis*, and *A celebesensis* was found to commence at around 50 mm TL (Kuroki et al. 2005 2006), which is considerably smaller than the metamorphosing size of the temperate *A. japonica*.

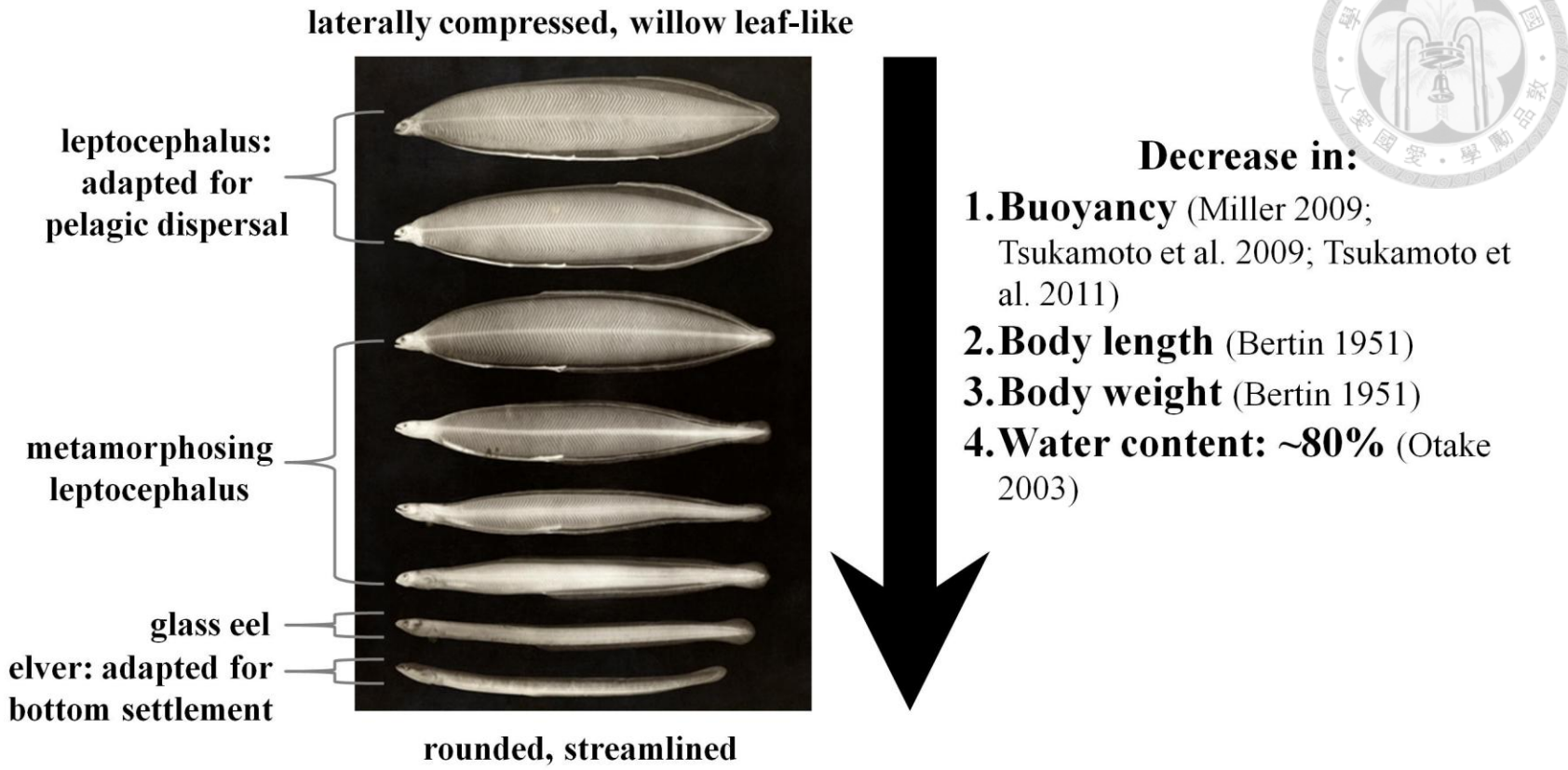


Fig. 46. Anguillid eel metamorphosis from leptocephalus to glass eel and some of the accompanying physiological changes. Eel metamorphosis picture courtesy of National Geographic Creative (http://www.natgeocreative.com/photography/1322898).

4.4 Delayed metamorphosis as a means of long-distance dispersal of the eel

At the end of their long transoceanic migration, *A. japonica* and *A. marmorata* leptocephali metamorphose into glass eels and invade coastal and inland habitats. Otolith microchemical studies revealed that after reaching coastal waters, glass eels may either migrate further inland and colonize freshwater habitats or stop their upstream migration and settle in seawater or estuaries (Tzeng et al. 2002, Arai et al. 2004, Daverat et al. 2006). The timing of metamorphosis by a leptocephalus into a glass eel and transport by oceanic currents are considered key determinants of the ultimate destination of eels (Cheng and Tzeng 1996, Tzeng 2003). In the present study, we found that the age of *A. marmorata* at metamorphosis from leptocephalus to glass eel (113.5 ± 13.0 d) was younger than in *A. japonica* (140.7 ± 13.6 d). Because metamorphosis triggers a behavioral switch from pelagic migration to bottom settlement, *A. japonica* leptocephali which arrive in Philippine waters are apparently too young to metamorphose and migrate towards estuaries so they continue drifting northwards or southwards. This must be the reason why Japanese eels are seldom found in the Philippines, while *A. marmorata* occurs in abundance (Tabeta et al. 1975, 1976). A similar scenario was also observed in American and European eels, for which differences in the duration of the leptocephalus stage and growth rates were the principal factors triggering segregative migration of these 2 species in the Atlantic Ocean (Wang and Tzeng 2000). The delay in metamorphosis of about 12~15 months in *A. anguilla* (McCleave 1993, Wang and Tzeng 2000) is necessary for its long-distance dispersal that includes a trans-Atlantic crossing. Similarly, it seems that *A. japonica* has developed a strategy to delay its metamorphosis from leptocephali to glass eels by reducing its growth rate, which enables it to migrate segregatively with *A. marmorata* and experience long-distance dispersal in East Asia. Its faster-growing and earlier-metamorphosing

leptocephali are recruited in Taiwan, while those that do not continue to drift towards eastern China and Japan. On the other hand, the faster-growing and earlier-metamorphosing leptocephali of *A. marmorata* are recruited earlier in the Philippines, while its slower-growing, delayed metamorphosing leptocephali disperse southward (via the Mindanao Current) and northward (via the KC). The difference in age at metamorphosis between *A. japonica* and *A. marmorata* ranged 18.5~39.9 d, and the delay in metamorphosis of 18.5~39.9 d is enough to allow *A. japonica* to be transported from North Luzon, the Philippines to further north in continental East Asia by the KC. Aside from these considerations, anomalies in the hydrology of the region should also be taken into account, because they might also influence the duration of larval drift and subsequently delay metamorphosis and affect recruitment. Anomalies such as El Niño and El Niño Southern Oscillation were found to affect current systems in the region. During El Niño years, the salinity front in the NEC region retreats southward, leading to a southward shift in the spawning grounds, causing poor recruitment (Kimura et al. 2001, Sugimoto et al. 2001, Kim et al. 2007, Han et al. 2009). During this period, leptocephali experience longer drift, slower growth rates, delayed metamorphosis, and ultimately delayed recruitment. But during non-El Niño years, the hydrology of the region changes with the season and these changes are more or less regular. Specimens examined in the present study were collected from different years during their peak recruitment seasons during non-El Niño years. Accordingly, the effects of environmental factors, such as El Niño events, on larval transportation and subsequently delayed metamorphosis from leptocephali to glass eels were not examined in this study and would be a good topic for future research and long-term studies.

4.5 The early growth rate affects dispersal range of leptocephali

The migratory segregation between *A. japonica* and *A. marmorata* in the northwestern Pacific can be further understood by examining their larval growth rates. Patterns of ontogenetic changes in otolith DGIs from the P to the otolith edge were found to be similar between *A. japonica* (Cheng and Tzeng 1996, Tzeng 2003) and *A. marmorata* (Table 7) (Fig. 26); however, otolith DGI widths were greater and increment numbers were fewer in *A. marmorata* than *A. japonica* (Tables 7, 9). This indicates that during the early stage of development, *A. marmorata* has faster otolith growth rates than *A. japonica*. Faster-growing leptocephali are able to metamorphose and are recruited earlier to estuaries in the Philippines, while slower-growing ones metamorphose and are recruited later to estuaries in Taiwan, eastern China, Korea, and Japan. In addition to this, a close linear relationship between ages at metamorphosis and recruitment in temperate and tropical eel species was observed (Marui et al. 2001), further suggesting that early-metamorphosing glass eels are recruited at younger ages. A similar phenomenon was also observed in other anguillid species like *A. celebesensis*, *A. bicolor bicolor*, *A. bicolor pacifica*, *A. australis*, *A. anguilla*, *A. rostrata*, and *A. dieffenbachi*. A reduced growth rate in *A. japonica* larvae prevents metamorphosis in synchrony with *A. marmorata,* despite their overlapping spawning sites and the same oceanic transport and migratory routes. Also, the slower growth rate of *A. japonica* during the leptocephalus stage and its longer duration compared to *A. marmorata* seem to be due to the longer transportation distance.

4.6 Spatial and temporal population genetic structure of the giant mottled eel *Anguilla marmorata* in the northwestern Pacific

The *A. marmorata* sample set in this study was used to determine the spatio-temporal population of the species in the northwestern Pacific in a separate study (Cheng et al. 2012). A total of 223 *A. marmorata* elvers from the Philippines (45), Taiwan (143) and Japan (35) were used to analyze the genetic variability and population genetic structure using 5 microsatellite loci and in order to eliminate the probability of species admixture with *A. luzonensis* in the samples due to their morphological resemblance during elver stage, all of the specimens were screened using species-specific mtDNA cytb PCR. The results showed that the samples analyzed was comprised of just a single species, *A. marmorata* and its population in the northwestern Pacific do not have any significant genetic differentiation (F_{ST} = 0.002; P > 0.05), even if the samples from Japan were collected 10 years ago. Hierarchical AMOVA also suggested that the population genetic structure among seasons in Taiwan was not significant (F_{ST} = 0.003; P > 0.05). This may be because of the year-round spawning behavior of the tropical eels. *Anguilla marmorata* just like *A. celebesensis* and *A. reinhardtii*, spawn all year round (Arai et al. 1999a, 2001a; Sugeha et al. 2001a; Shen and Tzeng, 2007a,b) in order to adapt to the low but continuous marine productivity in the tropical area. Semelparous spawning behavior and year-round spawning pattern can contribute to the population genetic differentiation if the individuals from the same area went back to the spawning ground at the same time. But because the environment is stable in the tropical areas, the signal to initiate spawning migration is not obvious and because of this, the year round spawning behavior of tropical eels will increase the possibility for inter-cohort or inter-locality gene flow. This scenario was also found in the tropical *A. reinhardtii* in Australia (Shen and Tzeng, 2007a). Also, because *A. marmorata* in the

northwestern Pacific was transported by the North Equation Current and Kuroshio Current, there is no known physical barrier or other current systems that affect the population genetic differentiation. But there might be another reason for their lack of spatial and temporal population genetic differentiation. In contrast to the non-panmictic temperate eel *A. japonica*, the shorter leptocephalus larval duration of the tropical eels contribute to their narrower distribution range and also one of the reasons for panmixia in *A. marmorata* population. The leptocephalus duration is the key factor influencing their recruitment timing and location (Cheng and Tzeng, 1996; Wang and Tzeng, 1998, 2000). The latitudinal distribution of *A. marmorata* is much narrower than that of *A. japonica* and is usually concentrated in the lower latitude. Eels from the same latitude have more chance of meeting one another in the spawning ground because the environmental signals to start spawning migration is similar. This may be the reason why it is more common to observe population differentiation in the temperate eels than the tropical eels (Tseng et al. 2006). Previous study suggested that *A. japonica* have a weak spatial population genetic differentiation (Tseng et al. 2001, 2003) indicating that this temperate eel species is non-panmictic and may have limited gene flow within species and population.

4.7 Migration behavior and habitat use of *Anguilla marmorata* in eastern Luzon, Philippines

In the past, the migratory histories of several anguillid eel species including *A. marmorata* have been studied using the trace elements in their otoliths, specifically strontium. The Sr:Ca ratio in the otoliths of those eel species varied greatly according to the time they spent in different habitats with different salinity gradient (freshwater, brackish water and seawater). Thus, the Sr:Ca ratios in the otoliths of the anguillid eels could help in determining whether or not they enter freshwater during the elver stage and remain in freshwater, brackish water or seawater environment until they reach the silver eel stage or whether they wander among different habitats with differing salinity regimes.

Previous studies (Shiao et al. 2003; Briones et al. 2007) indicated that *A. marmorata* in the Philippines and Taiwan has restricted migration behavior during the yellow eel stage. In the present study, beyond the elver check, the otolith Sr:Ca ratios of the samples collected from the wild maintained a value less than 4×10^{-3}, indicating that after migrating to the river, *A. marmorata* did not frequently inhabit high saline environment until capture. On the other hand, the otolith Sr:Ca ratios of the samples collected from an aquaculture farm varied greatly but might be reflecting the culture practice of the farm. The migratory behavior of *A. marmorata* from the Philippines and Taiwan is in contrast to that reported in Bonin Islands, Japan (Chino and Arai 2010a) wherein three patterns were found: (1) freshwater residence, (2) brackish water residence and (3) residence in freshwater after recruitment but returning to brackish water. This pattern was similar to the migration habit of *A. japonica*. Chino and Arai (2010a) reasoned that in the Bonin Islands, *A. marmorata* is the only freshwater eel species thus interspecific competition may not occur between (among) eel species so that they are present in all types of

habitats available. In the Philippines however, *A. marmorata* is only one of the several species of freshwater eels to occur in the country thus mechanisms such as habitat segregation and behavior differentiation exist to avoid interspecific competition for available food and space and attain maximum benefits. Meanwhile in Vietnam, *A. marmorata* coexisted with *A. bicolor pacifica* and may share the same niches, use the same demersal habitats and forage for the same prey (Arai et al. 2013) but instead of evolving to have a restricted distribution such as those exhibited in the Philippines and Taiwan to avoid interspecific competition, *A. marmorata* in Vietnam developed instead a diversified migratory behavior similar to that reported in Bonin Island with one minor difference. The 3rd pattern showed residence shifting between sea and brackish water with no freshwater life. Whether this flexible migration pattern exhibited by *A. marmorata* in Vietnam is a response to inter- and intra-species competition or a response to the varying productivity (carrying capacity) of the habitat, is not yet clear. These findings indicate that *A. marmorata* in some areas of its wide distribution range, have a flexible migration strategy with a high degree of behavioral plasticity similar to that of the other tropical eel species like *A. bicolor bicolor* (Chino and Arai 2010b) and *A. bicolor pacifica* (Arai et al. 2013) and temperate eel species like *A. anguilla* (Arai et al. 2006; Daverat et al. 2006), *A. australis* and *A dieffenbachii* (Arai et al. 2004), *A. japonica* (Kotake et al. 2003; Shiao et al. 2003; Tzeng et al. 2003; Chino and Arai 2009) and *A. rostrata* (Jessop et al. 2002). Migratory behavior of *A. marmorata* may differ among habitats in response to inter- and intra-specific competition after they recruit to coastal waters. Furthermore, flexible migratory behavior coupled with an ability to utilize and adapt to a wide range of salinity gradient and habitat might also help explain why *A. marmorata* has a very wide distribution range. However, in some areas where *A. marmorata* is just restricted to a specific

habitat (salinity) like in the Philippines and Taiwan, they are more susceptible to overfishing, continuous habitat degradation and environmental stresses.

5. CONCLUSION AND PERSPECTIVE

5.1 Species-specific geographic distribution

In the Hsiukuluan River, eastern Taiwan, the species composition of the recruiting anguillid eels was dominated by *A. marmorata* with *A. japonica* and *A. bicolor pacifica* as minor species, which greatly differs from what was previously reported in rivers of northern, western, and southern Taiwan where *A. japonica* dominates. The recruitment season of *A. marmorata* is mainly from early summer to autumn but can occur almost year round, while that of *A. japonica* is during winter. *Anguilla bicolor pacifica* mainly recruits during autumn. These results suggest that tropical eels have a unique geographical distribution and recruitment season which greatly differs from those of the temperate eel, *A. japonica.* Further investigations on the species composition of recruiting anguillid eels on a wider scale are warranted to validate the occurrence of *A. celebesensis* in the natural waters of Taiwan to be able to establish the number of anguillid eel species in the island. This information is essential for fishery regulation and management implementation.

5.2 Role of larval growth rate and metamorphosis timing in determining the geographical distribution of the anguillid eel

The larval growth rate and the metamorphosis timing may play an important role in the geographical distribution of the sympatric anguillid eel species *A. japonica* and *A. marmorata* in the northwestern Pacific during their drift from their overlapping spawning ground in the waters west of the Mariana Island via the NEC and the Kuroshio Current to their continental freshwater growth habitat. *Anguilla marmorata* grow faster and metamorphose earlier than *A. japonica* thus it can be found abundantly in the tropical Philippines and subtropical Taiwan but few in temperate China, Korea and Japan. On the contrary, the temperate eel *A. japonica* is abundant

beyond Taiwan and few or none can be found in the tropical Philippines. This indicates that the differences in their growth rate and the timing of metamorphosis from leptocephalus to glass eel are the key factors in determining the continental distribution of these two sympatric anguillid eel species. The delayed metamorphosis with reduced growth rate in *A. japonica* leptocephalus may be an evolutionary strategy for the temperate species to extend their distribution area from the tropical to the temperate region, farther north than the distribution range of *A. marmorata*. However, the present study is indicative not quantitative so further extensive sampling is needed in this oceanic region particularly during the later stages of larval migration as the leptocephali enter the NEC bifurcation region. Also, there is a similar lack of information about the larvae of these two eel species as they begin to metamorphose at the western margin of the subtropical gyre as they approach the Kuroshio Current. These issues indicated that future researches should focus on further samplings that cover a wide seasonal range and that emphasize on the later stages of the larval migration are needed to fill in critical gaps in the knowledge about the early life history stages of *A. japonica* and *A. marmorata* in the northwestern Pacific.

5.3. Migratory environmental history of *Anguilla marmorata* is different from that of *A. japonica*

The Sr:Ca ratios in the otoliths of *A. marmorata* from the Philippines revealed that its migratory environmental history is quite different from that reported from Japan and Vietnam. *Anguilla marmorata* in Taiwan and the Philippines is restricted to freshwater while those reported in Japan and Vietnam has a more flexible migratory behaviour, a pattern similar to the ones reported in *A. japonica* and other temperate eel species. Interspecific competition, environmental factors, the productivity of the environment and adaptation may play an important role in the habitat preference of *A. marmorata* throughout its species range. Because of its

restriction to just freshwater environment, *A. marmorata* in the Philippines and Taiwan are more susceptible to both heavy fishing pressure and continuous habitat degradation as compared to their counterpart with flexible migration pattern. Furthermore, since the juvenile eels analyzed in this study were all collected in the upper reaches of the river, samples from other parts of the river system (i.e. middle and lower reaches) should be analyzed as well to avoid sampling bias. Also, because of the archipelagic nature of the Philippines, the habitat use and life history of *the eels* might differ among location so further studies should be undertaken to determine its detailed migratory history and also of the other tropical eel species to elucidate their habitat.

REFERENCES

Amiard-Triquet C, JC Amiard, AC Andersen, P Elie, C Metayer. 1988. The eel (*Anguilla anguilla*) as a bioindicator of metal pollution: factor limiting its use. Water Science and Technology **19:** 1229-1232.

Aoyama J. 2009. Life history and evolution of migration in catadromous eels (genus *Anguilla*). Aqua-BioScience Monographs **2:** 1-42.

Arai T. 2002. Migratory history of fishes: present status and perspectives of the analytical methods. Ichthyological Research 49: 1-23.

Arai T, T Otake, K Tsukamoto. 1997. Drastic changes in otolith microstructure and microchemistry accompanying the onset of metamorphosis in Japanese eel *Anguilla japonica*. Marine Ecology Progress Series **161:** 17-22.

Arai T, D Limbong, T Otake, K Tsukamoto. 1999a. Metamorphosis timing and inshore migration of tropical eels, *Anguilla* spp., in the Indo-Pacific. Marine Ecology Progress Series **182:** 283-293.

Arai T, T Otake, D Limbong, K Tsukamoto. 1999b. Early life history and recruitment of the tropical eel, *Anguilla bicolor pacifica*, as revealed by otolith microstructure and microchemistry. Marine Biology **133:** 319-326.

Arai T, T Otake, DJ Jellyman, K Tsukamoto. 1999c. Differences in the early life history of the Australian short finned eel *Anguilla australis* from Australia and New Zealand, as revealed by otolith microstructure and microchemistry. Marine Biology **135:** 381-389.

Arai T, J Aoyama, L Daniel, K Tsukamoto. 1999a. Species composition and inshore migration of tropical eels, *Anguilla* spp., recruiting to the estuary of the Poigar River, Sulawesi Island. Marine Ecology Progress Series **188:** 299-303.

Arai T, D Limbong, K. Tsukamoto. 2000. Validation of otolith daily growth increments in the tropical eel *Anguilla celebesensis*. Canadian Journal of Zoology **78:** 1078-1084.

Arai T, D Limbong, T Otake, K Tsukamoto. 2001a. Recruitment mechanisms of tropical eels *Anguilla* spp. and implications for the evolution of oceanic migration in the genus *Anguilla*. Marine Ecology Progress Series **216:** 253-264.

Arai T, J Aoyama, S Ishikawa, MJ Miller, T Otake, T Inagaki, K Tsukamoto. 2001b. Early life history of tropical *Anguilla* leptocephali in the western Pacific Ocean. Marine Biology **138**: 887-895.

Arai T, M Marui, MJ Miller, K Tsukamoto. 2002a. Growth history and inshore migration of the tropical eel, *Anguilla marmorata,* in the Pacific. Marine Biology **140:** 309-316.

Arai T, M Marui, T Otake, K Tsukamoto. 2002b. Coastal migration of tropical eel, *Anguilla marmorata*, from Taiwanese and Japanese coasts. Fisheries Science **68:** 152-157.

Arai T, MJ Miller, K Tsukamoto. 2003. Larval duration of the tropical eel *Anguilla celebesensis* from Indonesian and Philippine coasts. Marine Ecology Progress Series **251:** 255-261.

Arai T, A Kotake, PM Lokinan, MJ Miller, K Tsukamoto. 2004. Evidence of different habitat use by New Zealand freshwater eels A*nguilla australis* and *A. dieffenbachi* as revealed by otolith microchemistry. Marine Ecology Progress Series **266:** 213-225.

Arai T, A Kotake, TK McCarthy. 2006. Habitat use by the European eel *Anguilla anguilla* in Irish waters. Estuarine, Coastal and Shelf Science 67: 569-578.

Arai T, N Chino, DQ Le. 2013. Migration and habitat use of the tropical eels *A. marmorata* and *A. bicolor pacifica* in Vietnam. Aquatic Ecology 47: 57-65.

August SM, BJ Hicks. 2008. Water temperature and upstream migration of glass eels in New

Zealand: implications of climate change. Environmental Biology of Fishes 81: 195-205.

Bertin L. 1951. Les anguilles. Payot, Paris.

Bertin L. 1956. Eels- a biological study. Cleaver-Hume Press, London.

Bonhommeau S, E Chassot, B Planque, A Knap, O Le Pape. 2008. Impact of climate on eel populations of Northern Hemisphere. Marine Ecology Progress Series **373:** 71-80.

Briand C, D Fatin, G Fontenelle, E Feunteun. 2003. Estuarine and fluvial recruitment of the European glass eel *Anguilla anguilla* in an exploited estuary. Fisheries Management and Ecology **10:** 377-384.

Briones AA, AV Yambot, JC Shiao, Y Iizuka, WN Tzeng. 2007. Migratory pattern and habitat use of tropical eels *Anguilla* spp. (Teleostei: Anguilliformes: Anguillidae) in the Philippines, as revealed by otolith microchemistry. The Raffles Bulletin of Zoology Supplement No. 14: 141-149.

Busch WDN, SJ Lary, CM Castilione, RP McDonald. 1998. Distribution and availability of Atlantic coast freshwater habitats for American eel (*Anguilla rostrata*). US Fish and Wildlife Service, Administrative report, Amherst, New York, pp 92-98.

Campana SE. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series 188: 263-297.

Campana SE, GA Chouinard, M Hanson, A Frechet, J Brattey, 2000. Otolith elemental fingerprints as biological tracers of fish stocks. Fisheries Research 46: 343-357.

Casselman JM. 2002. Effects of temperature, global extremes and climate change on year-class production of warmwater, coolwater and coldwater fishes in the Great Lakes Basin. *In* NA McGinn (ed) Proceedings of American Fisheries Society Symposium 32. American Fisheries Society, Besthada, p 39.

Castle PHJ, GR Williamson. 1974. On the validity of freshwater eel species *Anguilla ancestralis* Ege from Celebes. Copeia **2**: 659-670.

Castonguay M, PV Hodson, C Moriarty, KF Drinkwater, B Jessop. 1994. Is there a role of ocean environment in American and European eel decline? Fisheries Oceanography **3:** 197-203.

Chang KC, YS Han, WN Tzeng. 2007. Population genetic structure among intra-annual arrival waves of the Japanese eel *Anguilla japonica* in northern Taiwan. Zoological Studies **45:** 583-590.

Chen RT, CF Tsai, WN Tzeng. 2009. 16S and 28S rDNA sequences in phylogenetic analyses of freshwater prawns (*Macrobrachium* Bate, 1868) from Taiwan. Journal of Crustacean Biology **29:** 400-412.

Cheng PW, WN Tzeng. 1996. Timing of metamorphosis and estuarine arrival across the dispersal range of the Japanese eel *Anguilla japonica*. Marine Ecology Progress Series **131:** 87-96.

Cheng YL, HY Chen, WN Tzeng. 1994. Reappraisal of the importance of rainfall in affecting catches of *Anguilla japonica* elvers in Taiwan. Australian Journal of Marine and Freshwater Research **45:** 185-190.

Cheng CH, NJ Leander, KN Shen, WN Tzeng. 2012. Spatial and temporal population genetic structure of the giant mottled eel *Anguilla marmorata* in the Northwestern Pacific. Journal of the Fishery Society of Taiwan 39: 269-282.

Chino N, T Arai. 2009. Relative contribution of migratory type on the reproduction of migrating silver eels, *Anguilla japonica*, collected off Shikoku Island, Japan. Marine Biology 156: 661-668.

Chino N, T Arai. 2010a. Migratory history of the giant mottled eel (*Anguilla marmorata*) in the Bonin Islands of Japan. Ecology of Freshwater Fish 19: 19-25.

Chino N, T Arai. 2010b. Habitat use and habitat transitions in the tropical eel, *Anguilla bicolor bicolor*. Environmental Biology of Fishes 89: 571-578.

Chisnall BL, ML Martin, BJ Hicks. 2003. Effect of harvest on size, abundance and production of freshwater eels (*Australis australis* and *A. dieffenbachii*) in a New Zealand stream. *In* DA Dixon (ed) Biology, management and protection of catadromous eels. American fisheries Society, Besthada, pp 177-189.

Cieri MD, JD McCleave. 2001. Validation of daily otolith increments in glass-phase American eels *Anguilla rostrata* during estuarine residency. Journal of Experimental Marine Biology and Ecology **257:** 219-227.

Clevestam PD, M Ogonowski, NB Sjoberg, H Wickstrom. 2011. Too short to spawn? Implications of small body size and swimming distance on successful migration and maturation of the European eel *Anguilla anguilla*. Journal of Fish Biology **78:** 1073-1089.

Daverat F, KE Limburg, I Thibault, JC Shiao, JJ Dodson, F Caron, WN Tzeng, Y Iizuka, H. Wickstrom. 2006. Phenotypic plasticity of habitat use by three temperate eel species *Anguilla anguilla, A. japonica* and *A. rostrata*. Marine Ecology Progress Series **308:** 231-241.

Dekker W. 2003. Did lack of spawners cause the collapse of the European eel, *Anguilla anguilla*? Fisheries Management and Ecology **10:** 365-376.

Dekker W. 2008. Coming to grips with the eel stock slip-sliding away. *In* MG Schechter, NJ Leonard, eds. International Governance of Fisheries Ecosystems: Learning from the

past, finding solutions for the future. Bethesda, Maryland: American Fisheries Society, pp. 335-355.

Edeline E, S Dufour, P Elie. 2009. Proximate and ultimate control of eel continental dispersal. *In* G van den Thillart, S Dufour, J Cliff Rankin, eds. Spawning migration of the European eel. London: Springer, pp. 433-461.

Ege V. 1939. A revision of the genus *Anguilla* Shaw, a systematic, phylogenetic and geographical study. Dana Report **16:** 1-256.

Friedland KD, MJ Miller, B Knights. 2007. Oceanic changes in the Sargasso Sea and declines in the recruitment of European eel. ICES Journal of Marine Science **64:** 519-530.

Fukuda N. 2010. Inshore and upstream migration of Japanese eels in the Hamana Lake System, PhD thesis. University of Tokyo, Japan.

Graynoth E, D Jellyman, M Bonnett. 2008. Spawning escapement of female longfin eels. Ministry of Fisheries, New Zealand Fisheries Assessment Report 2008/7, Wellington.

Haenen O, V van Ginneken, M Engelsma, G van den Thillart. 2002. Impact of eel viruses on recruitment of European eel. *In* G van den Thillart, S Dufour, J Cliff Rankin, eds. Spawning migration of the European eel. London: Springer, pp. 387-400.

Han YS. 2010. The eel resource management. *In* International Symposium on Eel Culture and Trade. Nueva Ecija, the Philippines.

Han YS, CW Chang, JT He, WN Tzeng. 2001. Validation of the occurrence of short-finned eel *Anguilla bicolor pacifica* in natural waters of Taiwan. Acta Zoologica Taiwanica **12:** 9-19.

Han YS, CH Yu, HT Yu, CW Chang, IC Liao, WN Tzeng. 2002. The exotic American eel in Taiwan: ecological implications. Journal of Fish Biology **60:** 1608-1612.

Han YS, YT Chang, H Taraschewski, SL Chang, CC Chen, WN Tzeng. 2008. The swimbladder parasite *Anguillicola crassus* in native Japanese eels and exotic American eels in Taiwan. Zoological Studies **47**: 667-675.

Han YS, WN Tzeng. 2006. Use of sex ratio as a means of resource assessment for the Japanese eel *Anguilla japonica*: a case study in the Kaoping River, Taiwan. Zoological Studies 45: 255-263.

Han YS, YT Chang, WN Tzeng. 2009a. Variable habitat use by Japanese eel affects dissemination of swimbladder parasite *Anguillicola crassus*. Aquatic Biology 5: 143-147.

Han YS, WN Tzeng, IC Liao. 2009b. Time series analysis of Taiwanese catch data of Japanese glass eels *Anguilla japonica*: possible effects of the reproductive cycle and El Niño events. Zoological Studies **48:** 632-639.

Han YS, CL Hung, YF Liao, WN Tzeng. 2010. Population genetic structure of the Japanese eel *Anguilla japonica*: panmixia at spatial and temporal scales. Marine Ecology Progress Series **401:** 221-232.

Han YS, AV Yambot, H Zhang, CL Hung. 2012. Sympatric spawning but allopatric distribution of *Anguilla japonica* and *Anguilla marmorata*: temperature- and oceanic current-dependent sieving. Plos ONE **7:** e37484. doi:10.1371/journal.pone.0037484.

Haro A, W Richkus, K Whalen, A Hoar, WD Busch, S Lary, T Brush, D Dixon. 2000. Populations decline of the American eel: implications for future research and management. Fisheries **25**: 7-16.

Hoyle SD, DJ Jellyman. 2002. Longfin eels need reserves: modeling the effects of commercial harvest on stocks of New Zealand eels. Marine and Freshwater Research **53:** 887-895.

Ishikawa S. 1998. Molecular study on the population structure of *Anguilla marmorata*. PhD

thesis, University of Tokyo, Tokyo, Japan.

Ishikawa S, K. Tsukamoto, M. Nishida. 2004. Genetic evidence for multiple geographic populations of the giant mottled eel *Anguilla marmorata* in the Pacific and Indian oceans. Ichthyological Research **51:** 343-353.

IUCN. 2014. IUCN Red List of Threatened Species (ver. 2014.1). www.iucnredlist.org.

Jansen HM, HV Winter, MC Bruijs, HJG Polman. 2007. Just go with the flow? Route selection and mortality during downstream migration of silver eels in relation to river discharge. ICES Journal of Marine Science 64: 1437-1443.

Jellyman DJ. 1987. Review of the marine life history of Australasian temperate species of *Anguilla*. American Fisheries Society Symposium. **1:** 276-285.

Jellyman DJ, E Graynorth, RICC Francis, BL Chisnall, MP Beentjes. 2000. A review of the evidence for a decline in the abundance of longfinned eels (*Anguilla dieffenbachi*) in New Zealand. Final Research Report, Ministry of Fisheries Research Project EEL9802.

Kim H, S Kimura, A Shinoda, T Kitagawa, Y Sasai, H Sasaki. 2007. Effect of El Niño on migration and larval transport of the Japanese eel (*Anguilla japonica*). ICES Journal of Marine Science **64:** 1387-1395.

Kimura S, T. Inoue, T. Sugimoto. 2001. Fluctuation in the distribution of low-salinity water in the North Equatorial Current and its effect on the larval transport of the Japanese eel. Fisheries Oceanography **10:** 51-60.

Kirk RS. 2003. The impact of *Anguillicola crassus* on European eels. Fisheries Management and Ecology **10:** 385-394.

Knights B. 2003. A review of the possible impacts of long-term oceanic and climate changes

and fishing mortality on recruitment of anguillid eels of the Northern Hemisphere. Science of the Total Environment **310:** 237-244.

Kotake A, T Arai, T Ozawa, S Nojima, MJ Miller, K Tsukamoto. 2003. Variation in migratory history of Japanese eels, *Anguilla japonica*, collected in coastal waters of the Amakusa Islands, Japan, inferred from otolith Sr:Ca ratios. Marine Biology 142: 849-854.

Kuroki M, J Aoyama, MJ Miller, T Arai, HY Sugeha, G Minagawa, S Wouthuyzen, K Tsukamoto. 2005. Correspondence between otolith microstructural changes and early life history events in *Anguilla marmorata* leptocephali and glass eels. Coastal Marine Science **29:** 154-161.

Kuroki M, J Aoyama, MJ Miller, S Wouthuyen, T Arai, K Tsukamoto. 2006. Contrasting patterns of growth and migration of tropical anguillid leptocephali in the western Pacific and Indonesian Seas. Marine Ecology Progress Series **309:** 233-246.

Kuroki M, J Aoyama, MJ Miller, T Yoshinaga, A Shinoda, S Hagihara, K Tsukamoto. 2009. Sympatric spawning of *Anguilla marmorata* and *Anguilla japonica* in the western North Pacific Ocean. Journal of Fish Biology **74:** 1853-1865.

Leander NJ, KN Shen, RT Chen, WN Tzeng. 2012. Species composition and seasonal occurrence of recruiting glass eels (*Anguilla* spp.) in Hsiukuluan River, Eastern Taiwan. Zoological Studies **51:** 59-71.

Liao IC, SG Liao, WN Tzeng, CL Kuo. 1999. Investigation of leptocephalus by R/V Taiwan Fisheries Institute no. 1. Kaiyo Publisher, Tokyo, Kaiyo Monthly Special 18: 27-33.

Lin MH. 2001. Population dynamic of upstream migration in summer of *Anguilla marmorata* in Hsiukuluan River. Master's thesis, National Tsing Hua Univ., Hsinchu, Taiwan.

Lin YJ, Y Iizuka, WN Tzeng. 2009. Potential contributions by escaped cultured eels to the wild population of Japanese eel *Anguilla japonica* in the Kao-ping River. Journal of the Fisheries Society of Taiwan **36:** 179-189.

Lin YJ, WN Tzeng. 2008. Effect of shrimp net and cultured eels on the wild population of Japanese eel *Anguilla japonica* in the Kao-ping River, Taiwan. Journal of the Fisheries Society of Taiwan **35:** 61-37.

Lue X, S Fang, Y Zhang. 1999. Species and morphometric characters of upstream migrating elvers in Jiulongjiang Estuary. Journal of the Oceanography of Taiwan Strait. **18:** 191-194. (in Chinese with English abstract).

Lukas R, E Firing, P Hacker, PL Richardson, CA Collins, R Fine, R Gammon. 1991. Observations of the Mindanao Current during the Western Equatorial Pacific Ocean circulation study. Journal of Geophysical Research 96: 7089-7104.

Martin MH. 1995. Validation of daily growth increments in otoliths of *Anguilla rostrata* (Le Sueur) elvers. Canadian Journal of Zoology **73:** 208-211.

Marui M, T Arai, MJ Miller, DJ Jellyman, K Tsukamoto. 2001. Comparison of early life history between New Zealand temperate eels and Pacific tropical eels revealed by otolith microstructure and microchemistry. Marine Ecology Progress Series **213:** 273-284.

McCleave JD, RC Kleckner. 1987. Distribution of leptocephali of the catadromous *Anguilla* species in the western Sargasso Sea in relation to water circulation and migration. Bulletin of Marine Science **41:** 789-806.

McCleave JD, RC Kleckner, M Costonguay. 1987. Reproductive sympatry of American and European eels and implications for migration and taxonomy. American Fisheries Society Symposium **1:** 286-297.

McCleave JD. 1993. Physical and behavioral controls on the oceanic distribution and migration of leptocephali. Journal of Fish Biology **42:** 243-273.

McCleave JD. 2001. Simulation of the impact of dams and fishing weirs on reproductive potential of silver-phase American eels in the Kennebec River Basin, Maine. North American Journal of Fishery Management **21:** 592-605.

Miller MJ, N Mochioka, T Otake, K Tsukamoto. 2002. Evidence of a spawning area of *Anguilla marmorata* in the western North Pacific. Marine Biology **140:** 809-814.

Miller MJ. 2009. Ecology of anguilliform leptocephali; remarkable transparent fish larvae of the ocean surface layer. Aqua-BioScience Monographs **2:** 1-94.

Miller MJ, J Aoyama, K Tsukamoto. 2009. New perspectives on the early life history of tropical anguillid eels: implications for resource management. *In* JM Casselman, DK Cairns, eds. Eels at the edge: science, status and conservation concerns. American Fisheries Society, pp. 71-84.

Miller MJ, S Kimura, KD Friedland, B Knights, H Kim, DJ Jellyman, K Tsukamoto. 2009. Review of ocean-atmospheric factors in the Atlantic and Pacific Oceans influencing spawning and recruitment of anguillid eels. American Fishery Society Symposium **69**: 231-249.

Minegishi Y, J Aoyama, K Tsukamoto. 2008. Multiple population structure of the giant mottled eel, *Anguilla marmorata*. Molecular Ecology **17:** 3109-3122.

Minegishi Y, J Aoyama, JG Inoue, RV Azanza, K Tsukamoto. 2009. Inter-species and subspecific genetic divergences of freshwater eels, genus *Anguilla* including a recently described species, *A. luzonensis*, based on whole mitochondrial genome sequences. Coastal Marine Science 33: 64-77.

Moran RA. 1994. Adaptation and constraint in the complex life-cycles of animals. Annual Review of Ecological Systems **25:** 573-600.

Moriarty C, W Dekker. 1997. Management of the European eel *Anguilla anguilla*. Fishery Bulletin **15:** 1-110.

Nitani H. 1972. Beginning of the Kuroshio. In H Stommel, K Yashida (eds). Kuroshio: Its physical aspects. University of Tokyo Press, pp 129-163.

Otake T. 2003. Metamorphosis. *In* K Aida, K Tsukamoto, K. Yamauchi, eds. Eel Biology. Tokyo: Springer, pp. 61-74.

Otake T, T Ishii, M Nakahara, R Nakamura. 1994. Drastic changes in otolith strontium/calcium ratios in leptocephali and glass eels of Japanese eel *Anguilla japonica*. Marine Ecology Progress Series **112:** 189-193.

Otake T, T Ishii, M Nakahara, R Nakamura. 1997. Changes in otolith strontium:calcium ratios in metamorphosing *Conger myriaster* leptocephali. Marine Biology **128**: 565-572.

Ozawa T, O Tabeta, N Mochioka. 1989. Anguillid leptocephali from the western North Pacific east of Luzon, in 1988. Nippon Suisan Gakkaishi **55:** 627-632.

Palstra AP, DFM Heppener, VJT van Ginneken, C Szekely, G van den Thillart. 2006. Are dioxin-like contaminants responsible for the eel (*Anguilla anguilla*) drama? Naturwissenschaften 93: 145-148.

Panella G. 1980. Growth patterns in fish sagittae. *In* DC Rhoads, RA Lutz, eds. Skeletal growth of aquatic organisms. Plenum Press, New York, pp. 519-560.

Parker D, OLF Weyl, H Tarachewski. 2011. Invasion of a South African *Anguilla mossambica* (Anguillidae) population by the alien gill worm *Pseudodactylogyrus anguillae* (Monogenea). African Zoology 46: 371-377.

Richkus WA, K Whalen. 2000. Evidence for a decline in the abundance of the American eel, *Anguilla rostrata* (LeSueur), in North America since the early 1980s. Dana Report **12:** 83-97.

Robinet T, E Feunteun. 2002. Sublethal effects of exposure to chemical compounds: a cause for the decline in Atlantic eels? Ecotoxicology **11:** 265-277.

Sasal P, H Tarachewski, P Valade, H Grondin, S Wielgoss, F Moravec. 2008. Parasite communities in the eels of the island of Reunion (Indian Ocean): a lesson in parasite introduction. Parasitology Research 102: 1343-1350.

Shen KN, WN Tzeng. 2007a. Genetic differentiation among populations of the shortfinned eel *Anguilla australis* from East Australia and New Zealand. Journal of Fish Biology **70:** 177-190.

Shen, K. N. and W. N. Tzeng 2007b. Population genetic structure of the year-round spawning tropical eel *Anguilla reinhardtii* in Australia. Zoological Studies **46** (4): 331-453.

Shiao JC, WN Tzeng, A Collins, DJ Jellyman. 2001. Dispersal pattern of glass eel stage of *Anguilla australis* revealed by otolith growth increments. Marine Ecology Progress Series **219:** 241-250.

Shiao JC, Y Iizuka, CW Chang, WN Tzeng. 2003. Disparities in habitat use and migratory behavior between tropical eel *Anguilla marmorata* and temperate eel *A. japonica* in four Taiwanese rivers. Marine Ecology Progress Series **261:** 233-242.

Strubberg AC. 1913. The metamorphosis of elver as influenced by outward conditions. Meddelelser fra Kommissionen for Havunderseger Series Fiskeri, pp. 4-11.

Sugeha HY, T Arai, MJ Miller, D Limbong, K Tsukamoto. 2001a. Inshore migration of the tropical eels *Anguilla* spp. recruiting to the Poigar River estuary on north Sulawesi

Island. Marine Ecology Progress Series **221:** 233-243.

Sugeha HY, A Shinoda, M Marui, T Arai, K Tsukamoto. 2001b. Validation of otolith daily increments in the tropical eel *Anguilla marmorata*. Marine Ecology Progress Series **220:** 291-294.

Sugimoto T, S Kimira, K Tadokoro. 2001. Impact of El Niño events and climate regime shift on living resources in the western North Pacific. Progress in Oceanography **49:** 113-127.

Sures B, K Knopf. 2004. Parasites as a threat to freshwater eels? Science **304:** 209-211.

Szekely C, A Palstra, K Molnar, G van den Thillart. 2002. Impact of the swim-bladder parasite on the health and performance of European eels. *In* G van den Thillart, S Dufour, J Cliff Rankin, eds. Spawning migration of the European eel. London: Springer, pp. 201-226.

Tabeta O, N Mochioka. 2003. The glass eel. *In* K Aida, K Tsukamoto, K Yamauchi (eds). Eel Biology. Springer, Tokyo, pp 201-226.

Tabeta O, T Takai, I Matsui. 1975. Record of the Japanese eel from the Philippines. Bulletin of the Japanese Society of Scientific Fisheries **41**: 641-644.

Tabeta O, T Tanimoto, T Takai, I Matsui, T Imamura. 1976. Seasonal occurrence of anguillid elvers in Cagayan River, Luzon Island, the Philippines. Bulletin of the Japanese Society of Scientific Fisheries **42:** 421-426.

Tabeta O, K Tanaka, J Yamada, WN Tzeng. 1987. Aspects of the early life history of the Japanese eel *Anguilla japonica* determined from otolith microstructure. Bulletin of the Japanese Society of Scientific Fisheries **53:** 1727-1734.

Tanaka H, H Kagawa, H Ohta, K Okuzawa, K Hirose. 1995. The first report of eel larvae ingesting rotifers. Fisheries Science 61: 171-172.

Tatsukawa K. 2003. Eel resources in East Asia. *In* K Aida, K Tsukamoto, K. Yamauchi, eds.

Eel Biology. Tokyo: Springer, pp. 293-300.

Teng HY, YS Lin, CS Tzeng. 2009. A new *Anguilla* species and a reanalysis of the phylogeny of freshwater eels. Zoological Studies **48:** 808-822.

Tesch FW. 1977. The eel: biology and management of anguillid eels. London: Chapman and Hall.

Tesch FW. 2003. The eel, 3rd ed. Oxford, UK: Blackwell Science.

Tseng MC, SC Lee, WN Tzeng, 2001. Genetic variation of the Japanese eel *Anguilla japonica* based on microsatellite DNA. Journal of Taiwan Fisheries Research **9**: 137-147.

Tseng MC, WN Tzeng, SC Lee. 2003. Historical decline in the Japanese eel *Anguilla japonica* in Northern Taiwan inferred from temporal genetic variations. Zoological Studies **42**(4): 556-563.

Tseng MC, WN Tzeng, SC Lee. 2006. Population genetic structure of the Japanese eel *Anguilla japonica* in the northwest Pacific Ocean: evidence of non-panmictic populations. Marine Ecology Progress Series **308:** 221-230.

Tsukamoto K. 1990. Recruitment mechanism of the eel, *Anguilla japonica*, to the Japanese coast. Journal of Fish Biology **36:** 659-671.

Tsukamoto K, Y Yamada, A Okamura, T Kaneko, H Tanaka, MJ Miller, N Horie, N Mikawa, T Utoh, S Tanaka. 2009. Positive buoyancy in eel leptocephali: an adaptation for life in the ocean surface layer. Marine Biology **156:** 835-846.

Tsukamoto K, S Chow, T Otake, H Kurogi, N Mochioka, MJ Miller, J Aoyama, S Kimura, S Watanabe, T Yoshinaga, A Shinoda, M Kuroki, M Oya, T Watanabe, K Hata, S Ijiri, Y Kazeto, K Nomura, H Tanaka. 2011. Oceanic spawning ecology of freshwater eels in the western North Pacific. Nature Communications **2:** 179.

Tzeng WN. 1982. New record of the elver, *Anguilla celebesensis* Kaup, from Taiwan. Chinese Bioscience **19:** 57-66. (in Chinese with English abstract).

Tzeng WN. 1983a. Species identification and commercial catch of the anguillid elvers from Taiwan. China Fishery Monthly **366:** 16-23. (in Chinese with English abstract)

Tzeng WN. 1983b. Seasonal and long-term changes of the catch of anguillid elvers in Taiwan. Journal of the Fishery Society of Taiwan **10:** 1-7 (in Chinese with English abstract)

Tzeng WN. 1984a. Dispersion and upstream migration of marked anguillid eel, *Anguilla japonica* elvers in the estuary of Shuang-Chi River, Taiwan. Bulletin of the Japanese Society of Fisheries Oceanography **45:** 10-20.

Tzeng WN. 1984b. An estimate of the exploitation rate of *Anguilla japonica* elvers immigrating into the coastal waters of Shuang-Chi River, Taiwan. Bulletin of the Institute of Zoology Academia Sinica **23:** 173-180.

Tzeng WN. 1985. Immigration timing and activity rhythms of the eel *Anguilla japonica* elvers in the estuary of northern Taiwan, with emphasis on environmental influences. Bulletin of the Japanese Society of Fisheries Oceanography **47-48:** 11-28.

Tzeng WN. 1990. Relationship between growth rate and age at recruitment of *Anguilla japonica* elvers in a Taiwan estuary as inferred from otolith growth increments. Marine Biology **107:** 75-81.

Tzeng WN. 1995. Migratory history recorded in otoliths of the Japanese eel, *Anguilla japonica*, elvers as revealed from SEM and WDS analysis. Zoological Studies 34 (Supplement 1**):** 234-236.

Tzeng WN. 1996. Effects of salinity and ontogenetic movements on strontium: calcium ratios in the otoliths of the Japanese eel, *Anguilla japonica* Temminck and Schlegel. Journal of

Experimental Marine Biology and Ecology **199:** 111-122.

Tzeng WN. 1996. Short-and long-term fluctuations in catches of elvers of the Japanese eel *Anguilla japonica* in Taiwan. *In* DA Hancock, DC Smith, A Grant, JP Beumer, eds. Developing and sustaining world fisheries resources: the state of science and management. Second World Fisheries Congress Proceedings. Victoria, Australia: CSIRO, pp. 85-89.

Tzeng WN. 2003. The processes of inshore migration of the Japanese eel *Anguilla japonica* as revealed by otolith microstructure. *In* K Aida, K Tsukamoto, K Yamauchi, eds. Eel Biology. Tokyo: Springer, pp. 181-190.

Tzeng WN. 2006. Surface water temperature as a potential cause of delayed arrival of the Japanese eel *Anguilla japonica* elvers on the coast of Taiwan. Journal of the Fishery Society of Taiwan **33:** 115-126.

Tzeng WN, CW Chang. 2001. Stock status and management prospect of the freshwater eel *Anguilla* spp. in Taiwan. Journal of Taiwan Fishery Research **9:** 251-258.

Tzeng WN, PW Cheng, FY Lin. 1995. Relative abundance, sex ratio and population structure of the Japanese eel *Anguilla japonica* in the Tanshui River system of northern Taiwan. Journal of Fish Biology **46:** 183-201.

Tzeng WN, O Tabeta. 1983. First record of the short-finned eel *Anguilla bicolor pacifica* elvers from Taiwan. Bulletin of the Japanese Society of Fisheries Oceanography **49:** 27-32.

Tzeng WN, YC Tsai. 1992. Otolith microstructure and daily age of *Anguilla japonica* Temminck and Schlegel elvers from the estuaries of Taiwan with reference to unit stock and larval migration. Journal of Fish Biology **40:** 845-857.

Tzeng WN, YC Tsai. 1994. Changes in otolith microchemistry of the Japanese eel, *Anguilla*

japonica, during its migration from the ocean to the rivers of Taiwan. Journal of Fish Biology **45:** 671-684.

Tzeng WN, JC Shiao, Y Iizuka. 2002. Use of otolith Sr:Ca ratios to study the riverine migratory behaviours of Japanese eel *Anguilla japonica*. Marine Ecology Progress Series **245:** 213-221.

Tzeng WN, YH Tseng, YS Han, CC Hsu, CW Chang, E Di Lorenzo, CH Hsieh. 2012. Evaluation of multi-scale climate effects on annual recruitment levels of the Japanese eel, *Anguilla japonica*, to Taiwan. PLOS ONE 7:e30805.

Umezawa A, K Tsukamoto, O Tabeta, H Yamakawa. 1989. Daily growth increments in the larval otolith of the Japanese eel, *Anguilla japonica*. Ichthyological Research **35:** 440-444.

van Ginneken V, B Ballieux, R Willemze, K Coldenhoff, E Lentjes, E Antonnissen, O Haenen, G van den Thillart. 2005. Hematology patterns of migrating European eels and the role of EVEX virus. Comparative Biochemistry and Physiology Part C **140:** 97-102.

van Ginneken V, M Bruijs, T Murk, A Palstra, G van den Thillart. 2009. The effects of PCBs on the spawning migration of the European silver eel. *In* G van den Thillart, S Dufour, J Cliff Rankin, eds. Spawning migration of the European eel. London: Springer, pp. 365-386.

Verreault G, P Dumont, Y Mailhot. 2004. Habitat losses and anthropogenic barriers as a cause of population decline for American eel (Anguilla rostrata) in St. Lawrence watershed, Canada. *In* 2004 ICES Annual Science Conference, Vigo, 22-25 Spetember 2004. ICES, Copenhagen.

Wang CH, WN Tzeng. 1998. Interpretation of geographic variation in size of American eel,

Anguilla rostrata (Le Sueur), elvers on the Atlantic coast of North America by their life history and otolith ageing. Marine Ecology Progress Series **168:** 35-43.

Wang CH, WN Tzeng. 2000. The timing and growth rates of American and European eel leptocephali: a mechanism of larval segregative migration. Fisheries Research **46:** 191-205.

Watanabe S, J Aoyama, MJ Miller, S Ishikawa, E Feunteun, K Tsukamoto. 2008. Evidence of population structure in the giant mottled eel, *Anguilla marmorata*, using total number of vertebrae. Copeia **2008:** 680-688.

Watanabe S, J Aoyama, K Tsukamoto. 2009. A new species of freshwater eel *Anguilla luzonensis* (Teleostei: Anguillidae) from Luzon Island of the Philippines. Fisheries Science **75:** 387-392.

Watanabe S, MJ Miller, J Aoyama, K. Tsukamoto. 2009. Morphological and meristic evaluation of the population structure of *Anguilla marmorata* across its range. Journal of Fish Biology **74:** 2069-2093.

Zenimoto K, T Kitagawa, S Miyazaki, Y Sasai, S Kimura. 2009. The effects of seasonal and interannual variability of oceanic structure in the western Pacific North Equatorial Current on larval transport of the Japanese eel *Anguilla japonica*. Journal of Fish Biol. **74:** 1878-1890.

APPENDIX

Appendix 1. Fisheries Administrative Order 242 of the Philippines

Republic of the Philippines
DEPARTMENT OF AGRICULTURE
Office of the Secretary
Elliptical Road, Diliman, Quezon City

FISHERIES ADMINISTRATIVE ORDER NO. 242
Series of 2012

SUBJECT : Reinstating the ban on the export of elvers

Pursuant to Sections 61.b, 99 and 107 of Republic Act 8550, Fisheries Administrative Order No. 159 series of 1986 is hereby revoked and the ban on the export of elvers is hereby reinstated subject to the following provisions:

Section 1. Definition of terms. – These terms for purposes of this Order shall be construed as follows:

1. Eel fingerling – means juvenile form of all eels or snake-like fish more than five (5) centimeters but not exceeding fifteen (15) centimeters in length;

2. Eel fry – means post larval forms of all eels or snake-like fish not exceeding five (5) centimeters in length, with glass-like transparency, and also called glass eels;

3. Eel species – pertains to species of fish under the Family Anguillidae, specifically these species reported to occur in the country: *Anguilla luzonensis, A. celebensis, A. malgumora, A. marmorata, A. japonica, A. bicolor bicolor, A. bengalensis bengalensis* and *A. australis australis;*

4. Elvers – means young eels which refer to eel fry or eel fingerlings;

5. Export – means to send or ship out of the country.

Section 2. Prohibition. - It shall be unlawful for any person, association or corporation to export or cause to be exported fry and fingerlings of eel species as defined. *Provided*, however, that the Secretary of Agriculture, pursuant to an approved scientific research and upon the recommendation of the Director of the Bureau of Fisheries and Aquatic Resources (BFAR), may grant a special permit to export eel fry or fingerlings of not more than one (1) kilo live weight for scientific and/or educational purposes, subject to such conditions as the Secretary may deem wise to impose.

Section 3. Penalty. - Any violation of this Order shall subject the offender to imprisonment of eight (8) years, confiscation of the same or a fine equivalent to double the export value of the same upon the discretion of the court, and revocation of the fishing and/or export accreditation/permit.

Section 4. Repealing clause. - All orders, rules and regulations or parts thereof inconsistent with the provisions of this Order are hereby repealed.

Section 5. Effectivity. – This Order shall take effect fifteen (15) days after its publication in a newspaper of general circulation and upon acknowledgement by the Office of the National Administrative Registry (ONAR).

Issued this 10th day of April 2012, at Quezon City, Philippines.

PROCESO J. ALCALA
Secretary

DEPARTMENT OF AGRICULTURE
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Recommended by:

ATTY. ASIS G. PEREZ
Director, BFAR

SALVADOR S. SALACUP
Undersecretary for Fisheries and
NFARMC Chairman

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Appendix 2. Papers, abstracts and presentations related to this dissertation

1. Published manuscripts

Leander NJ, Shen KN, Chen RT and Tzeng WN (2012). Species composition and seasonal occurrence of recruiting glass eels (*Anguilla* spp.) in the Hsiukuluan River, Eastern Taiwan. Zoological Studies 51 (1): 59-71.

Leander NJ, Tzeng WN, Yeh NT, Shen KN and Han YS (2013). Effects of metamorphosis timing and the larval growth rate on the latitudinal distribution of sympatric freshwater eels, *Anguilla japonica* and *A. marmorata*, in the western North Pacific. Zoological Studies 52 (1): 30-45.

2. Conference paper (abstracts)

Leander NJ, Shen KN, Yeh NT and Tzeng WN (2012). Can age at metamorphosis and early growth rate influence the latitudinal distribution of *Anguilla japonica* and *A. marmorata*? 6th World Fisheries Congress, May 7-11, 2012, Edinburgh International Convention Centre, Edinburgh, Scotland.

Leander NJ and Tzeng WN (2011). Metamorphosis timing: a mechanism for segregative recruitment in *Anguilla japonica* and *Anguilla marmorata* in the northwestern Pacific? 9th Asian Fisheries and Aquaculture Forum, April 21-25, 2011, Shanghai Ocean University, Shanghai, China.

Leander NJ, Shen KN, Chen RT and Tzeng WN (2010). *Anguilla* spp. recruiting to Hsiukuluan River, eastern Taiwan: Taxonomy, species composition and seasonal occurrence. 2010 Annual Meeting of The Fisheries Society of Taiwan, December 11, 2010, National Taiwan Ocean University, Keelung, Taiwan.

Leander NJ, Han YS, Chen RT, and Tzeng WN (2010). Species composition and seasonal occurrence of tropical eel (*Anguilla* spp.) in the estuary of Hsiukuluan River, with reference to the revision of the Anguillid eel species found in Taiwan. The First Cross-Strait Workshop on Marine Biodiversity, November 4-7, 2010, Xiamen, China.